JAN 89614

SEARCH REQUEST FORM

Scientific and Technical Information Center

Requester's Full Name: _R Gitomer_ Examiner #: _69680_ Date: _3/21/03_

Art Unit: _1651_ Phone Number 308-0732 Serial Number: _09/039,957_

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_1011_

If more than one search is submitted, please prioritize searches in order of need.

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Please provide a detailed statement of the search topic, and describe as specifically as possible the subject matter to be searched. Include the elected species or structures, keywords, synonyms, acronyms, and registry numbers, and combine with the concept or utility of the invention. Define any terms that may have a special meaning. Give examples or relevant citations, authors, etc, if known. Please attach a copy of the cover sheet, pertinent claims, and abstract.

Title of Invention: _____

Inventors (please provide full names): _____

_____

Earliest Priority Filing Date: _____

For Sequence Searches Only Please include all pertinent information (parent, child, divisional, or issued patent numbers) along with the appropriate serial number.

JAN

Jan Delaval
Reference Librarian
Biotechnology & Chemical Library
CM1 1E07 – 703-308-4498
jan.delaval@uspto.gov

****

STAFF USE ONLY	Type of Search	Vendors and cost where applicable
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Date Searcher Picked Up: _4/7/03_	Bibliographic ✓	Dr.Link _____
Date Completed: _4/8/03_	Litigation _____	Lexis/Nexis _____
Searcher Prep & Review Time: _____	Fulltext _____	Sequence Systems _____
Clerical Prep Time: _30_	Patent Family _____	WWW/Internet _____
Online Time: _+ 1 ω_	Other _____	Other (specify) _____ ✓

PTO-1590 (8-01)

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L89   ANSWER 1 OF 5  HCAPLUS  COPYRIGHT 2003 ACS
AN    2002:869478  HCAPLUS
DN    137:348811
TI    Staining agents and protocols for characterization of malignant cells in
      culture
IN    Naus, Gregory J.; Kornblith, Paul L.; Burholt, Dennis R.; Meyer,
      Michael P.
PA    USA
SO    U.S. Pat. Appl. Publ., 8 pp.
      CODEN: USXXCO
DT    Patent
LA    English
IC    ICM  G01N033-53
      ICS  C07K016-00
NCL   435007100
CC    9-10 (Biochemical Methods)
      Section cross-reference(s): 1, 8, 15
FAN.CNT 3
      PATENT NO.         KIND  DATE          APPLICATION NO.  DATE
      --------------     ----  --------      ---------------  --------
PI    US 2002168679      A1    20021114      US 1998-95993    19980611
      US 2001051353      A1    20011213      US 1998-189310   19981110
      US 6416967         B2    20020709
PRAI  US 1996-679056     A2    19960712
      US 1998-39957      A2    19980316
      US 1998-95993      A2    19980611
AB    An improved system for screening a multiple of candidate therapeutic or
      chemotherapeutic agents for efficacy as to a specific patient, in which a
      tissue sample from the patient is harvested, cultured and sep. exposed to
      a plurality of treatments and/or therapeutic agents for the purpose of
      objectively identifying the best treatment or agent for the particular
      patient.  Specific method innovations such as tissue sample prepn.
      techniques render this method practically as well as theor. useful.  The
      identity of the malignant cells in culture is advantageously confirmed
      using binding reagents/staining systems specific for epithelial cells,
      since carcinomas are ubiquitously epithelial in nature.  Cells of interest
      and thus confirmed as epithelial/carcinomal may then be assayed for
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sensitivity to an infinite variety of malignancy treating agents including chemotherapeutic agents, radiation, immunotherapy, and so on.
ST   malignant human carcinoma epithelial cell culture staining agent binder; carcinomal epithelial cell system chemotherapeutic antitumor radiation immunotherapy sensitivity
IT   Keratins
       RL: BSU (Biological study, unclassified); BIOL (Biological study)
         (AE1 and AE3; staining agents and protocols for characterization of
         malignant cells in culture)
IT   Antibodies
       RL: BUU (Biological use, unclassified); BIOL (Biological study); USES
       (Uses)
         (CAM; staining agents and protocols for characterization of malignant
         cells in culture)
IT   Antibodies
       RL: BUU (Biological use, unclassified); BIOL (Biological study); USES
       (Uses)
         (anti-cytokeratin; staining agents and protocols for characterization
         of malignant cells in culture)
IT   Antibodies
       RL: BUU (Biological use, unclassified); BIOL (Biological study); USES
       (Uses)
         (anti-epithelial-membrane-antigen; staining agents and protocols for
         characterization of malignant cells in culture)
IT   Cell
         (cancer; staining agents and protocols for characterization of
         malignant cells in culture)
IT   Mucins
       RL: BSU (Biological study, unclassified); BIOL (Biological study)
         (gene MUC1; staining agents and protocols for characterization of
         malignant cells in culture)
IT   **Antitumor agents**
       Chemotherapy
       Immunotherapy
       Radiotherapy
         (improved system for screening of; staining agents and protocols for
         characterization of malignant cells in culture)
IT   Antibodies
       RL: BUU (Biological use, unclassified); BIOL (Biological study); USES
       (Uses)
         (monoclonal, AE1 cytokeratin-specific; staining agents and protocols
         for characterization of malignant cells in culture)
IT   Antibodies
       RL: BUU (Biological use, unclassified); BIOL (Biological study); USES
       (Uses)
         (monoclonal, AE1/AE3 cytokeratin-specific; staining agents and
         protocols for characterization of malignant cells in culture)
IT   Antibodies
       RL: BUU (Biological use, unclassified); BIOL (Biological study); USES
       (Uses)
         (monoclonal, AE3 cytokeratin-specific; staining agents and protocols
         for characterization of malignant cells in culture)
IT   Antibodies
       RL: BUU (Biological use, unclassified); BIOL (Biological study); USES
       (Uses)
         (monoclonal, epithelial membrane antigen-specific; staining agents and
         protocols for characterization of malignant cells in culture)
IT   **Animal tissue culture**
       Binders
         Drug screening
         Epithelium
       Human
       Staining, biological

Transformation, neoplastic
(staining agents and protocols for characterization of malignant cells
in culture)
IT 9003-99-0D, Peroxidase, conjugate with streptavidin 9013-20-1D,
Streptavidin, conjugate with peroxidase 78849-24-8, Chromagen
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES
(Uses)
(staining agents and protocols for characterization of malignant cells
in culture)

L89   ANSWER 2 OF 5 HCAPLUS COPYRIGHT 2003 ACS
AN   2002:315130 HCAPLUS
DN   136:306444
TI   Method for tandem genomic/proteomic analyses of proliferating cells
IN   **Kornblith, Paul L.**; Donovan-Peluso, Maryann
PA   **Precision Therapeutics**, Inc., USA
SO   PCT Int. Appl., 63 pp.
      CODEN: PIXXD2
DT   Patent
LA   English
IC   ICM C12Q
CC   **9-16 (Biochemical Methods)**
      Section cross-reference(s): 1, 3, 14
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2002033117	A2	20020425	WO 2001-US32540	20011018
WO 2002033117	A3	20020627		

      W: AE, AG, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH,
          CN, CO, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EC, EE, EE, ES,
          FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG,
          KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
          MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL,
          TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY,
          KG, KZ, MD, RU
      RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY,
          DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF,
          BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
PRAI US 2000-691492   A   20001018
     US 2001-273281P   P   20010302
AB   The invention concerns a novel process for producing data characterizing
      nucleic acids in proliferating cells using a tandem protocol for
      simultaneously building and expanding genomics/proteomics cancer databases
      while coordinating present and future genomic/proteomic individual patient
      assays by collecting a tissue sample including the proliferating cells;
      mech. dividing the sample into cohesive multicellular particulates, and
      growing and analyzing the resulting cells. Sets of genetic data can be
      analyzed along with sets of corresponding clin. data, including phenotypic
      data, to form profiles that can aid in identifying proliferative diseases
      and in prognoses. A method for diagnosing proliferative diseases using
      the described assay methods is also provided. Lastly, a computing device
      is provided which permits searching anal. and entry of the genetic data,
      the corresponding clin. data and/or profiles.
ST   genome proteome cancer database diagnosis nucleic acid DNA RNA
IT   Gene, animal
      RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
      (HSV-TK; method for tandem genomic/proteomic analyses of proliferating
      cells)
IT   Mammary gland
      Ovary, neoplasm
      (carcinoma; method for tandem genomic/proteomic analyses of
      proliferating cells)
IT   Neuroglia

         (glioblastoma; method for tandem genomic/proteomic analyses of
         proliferating cells)
IT   Alleles
         Animal cell
         Animal tissue
         Animal tissue culture
      Apoptosis
      Brain, neoplasm
      Cell proliferation
      Chemotherapy
      Computers
      Cryopreservation
      DNA microarray technology
      Databases
      Diagnosis
         Drug screening
      Fluorescent substances
      Gene therapy
      Genetic polymorphism
      Genome
      Human
      Immunotherapy
      Luminescence, bioluminescence
      Neoplasm
      Phenotypes
      Process automation
      Prognosis
      Radiotherapy
      Staining, biological
         (method for tandem genomic/proteomic analyses of proliferating cells)
IT   DNA
      Nucleic acids
      RNA
      RL: ANT (Analyte); DGN (Diagnostic use); PRP (Properties); ANST
      (Analytical study); BIOL (Biological study); USES (Uses)
         (method for tandem genomic/proteomic analyses of proliferating cells)
IT   Probes (nucleic acid)
      RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)
         (method for tandem genomic/proteomic analyses of proliferating cells)
IT   Reagents
      RL: ARU (Analytical role, unclassified); ANST (Analytical study)
         (method for tandem genomic/proteomic analyses of proliferating cells)
IT   Gene, animal
      RL: BSU (Biological study, unclassified); BIOL (Biological study)
         (method for tandem genomic/proteomic analyses of proliferating cells)
IT   Proteome
      RL: BSU (Biological study, unclassified); BIOL (Biological study)
         (method for tandem genomic/proteomic analyses of proliferating cells)
IT   Hormones, animal, biological studies
      RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
         (method for tandem genomic/proteomic analyses of proliferating cells)
IT   **Lymphocyte**
         (natural killer cell; method for tandem genomic/proteomic analyses of
         proliferating cells)
IT   Disease, animal
         (proliferative; method for tandem genomic/proteomic analyses of
         proliferating cells)
IT   Kidney, neoplasm
         (renal cell carcinoma; method for tandem genomic/proteomic analyses of
         proliferating cells)
IT   56-65-5, ATP, uses   56-81-5, Glycerol, uses   67-68-5, DMSO, uses
      69-65-8, Mannitol
      RL: NUU (Other use, unclassified); USES (Uses)

```
            (method for tandem genomic/proteomic analyses of proliferating cells)
IT    7727-37-9, Nitrogen, uses
            RL: NUU (Other use, unclassified); PRP (Properties); USES (Uses)
            (method for tandem genomic/proteomic analyses of proliferating cells)
IT    82410-32-0, Ganciclovir
            RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
            (method for tandem genomic/proteomic analyses of proliferating cells)


L89   ANSWER 3 OF 5  HCAPLUS  COPYRIGHT 2003 ACS
AN    2001:906155  HCAPLUS
DN    136:17691
TI    Method for using multicellular particulates to analyze malignant or
      hyperproliferative tissue
IN    Kornblith, Paul L.
PA    USA
SO    U.S. Pat. Appl. Publ., 24 pp., Cont.-in-part of U. S. 5,728,541.
      CODEN: USXXCO
DT    Patent
LA    English
IC    ICM  C12Q001-02
      ICS  C12Q001-24; C12Q001-18
NCL   435029000
CC    9-4 (Biochemical Methods)
      Section cross-reference(s): 1, 14
FAN.CNT 3
      PATENT NO.         KIND  DATE          APPLICATION NO.   DATE
      --------------     ----  --------      ---------------   --------
PI    US 2001051353      A1    20011213      US 1998-189310    19981110
      US 6416967         B2    20020709
      US 5728541         A     19980317      US 1996-679056    19960712
      US 2002168679      A1    20021114      US 1998-95993     19980611
      US 2002192638      A1    20021219      US 2002-205887    20020726
PRAI  US 1996-679056     A2    19960712
      US 1998-39957      A2    19980316
      US 1998-95993      A2    19980611
      US 1998-40161      A1    19980317
AB    The invention concerns a comprehensive and integrated system for
      monitoring (identifying, tracking and analyzing) an individual patient's
      malignancy through the duration of a malignancy as to a specific patient
      is provided.  The method of the present invention allows for initial
      identification of a malignancy, identification of malignancy-specific
      cellular or secretory markers, identification of cellular or secreted
      markers indicative of complications, study of the invasiveness and
      aggressiveness of the malignancy, study of the growth rate of the
      malignancy, study of the effect of therapies on the malignancy as compared
      to control cells of the same patient (chemo-sensitivity vs. toxicity) and
      the identification of a therapeutic index (i.e., the ratio of
      chemo-sensitivity:toxicity), study of tumor morphol. and study of histol.,
      cytochem. and immunocytochem. markers.
ST    tumor malignancy therapy immunoassay histol cytochem drug screening
      resistance
IT    Neoplasm
          (biopsy; method for using multicellular particulates to
          analyze malignant or hyperproliferative tissue)
IT    Gene, animal
      RL: ANT (Analyte); DGN (Diagnostic use); ANST (Analytical study); BIOL
      (Biological study); USES (Uses)
          (cancer related, mutation in; method for using multicellular
          particulates to analyze malignant or hyperproliferative tissue)
IT    Neuroglia
          (glioblastoma; method for using multicellular particulates to analyze
          malignant or hyperproliferative tissue)
IT    Immunoassay
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(immunocytochem.; method for using multicellular particulates to
analyze malignant or hyperproliferative tissue)
IT Drug resistance   .
    Phenotypes
        (marker for; method for using multicellular particulates to analyze
        malignant or hyperproliferative tissue)
IT **Mesothelium**   .
        (mesothelioma; method for using multicellular particulates to analyze
        malignant or hyperproliferative tissue)
IT **Animal cell**
    Animal tissue
    Animal tissue culture
    Brain, neoplasm
    Cell proliferation
    Chemotherapy
    Computer application
    Culture media
    Drug resistance
    Drug screening
    Epithelium
    Gene therapy
    Genetic methods .
    Genotypes
    Histochemistry   `
    Human
    Immunotherapy
    Kidney, neoplasm
    Microtiter plates
    Neoplasm
    Ovary, neoplasm
    Process automation
    Radiotherapy
    Thrombosis
    Tumor markers
        (method for using multicellular particulates to analyze malignant or
        hyperproliferative tissue)
IT Estrogen receptors
    Progesterone receptors
    RL: ANT (Analyte); DGN (Diagnostic use); ANST (Analytical study); BIOL .
    (Biological study); USES (Uses)
        (method for using multicellular particulates to analyze malignant or
        hyperproliferative tissue)
IT **Lymphocyte**
        (natural killer cell; method for using multicellular particulates to
        analyze malignant or hyperproliferative tissue)
IT Mammary gland
        (neoplasm; method for using multicellular particulates to analyze
        malignant or hyperproliferative tissue)
IT Mutation
    · (of cancer related genes; method for using multicellular particulates
        to analyze malignant or hyperproliferative tissue)
IT Gene, animal
    RL: ANT (Analyte); DGN (Diagnostic use); ANST (Analytical study); BIOL
    (Biological study); USES (Uses)
        (oncogene; method for using multicellular particulates to analyze
        malignant or hyperproliferative tissue)
IT Drug tolerance
        (therapeutic index; method for using multicellular particulates to
        analyze malignant or hyperproliferative tissue)
IT Hormones, animal, biological studies
    RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
        (therapy; method for using multicellular particulates to analyze
        malignant or hyperproliferative tissue)

IT   Cell morphology
          (tumor; method for using multicellular particulates to analyze
          malignant or hyperproliferative tissue)
IT   302-79-4, Retinoic acid   10540-29-1, Tamoxifen   33069-62-4, Taxol
      41575-94-4, Carboplatin
      RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
          (method for using multicellular particulates to analyze malignant or
          hyperproliferative tissue)

L89   ANSWER 4 OF 5 HCAPLUS COPYRIGHT 2003 ACS
AN   **1998:71095** HCAPLUS
DN   **128:97698**
TI   Precise efficacy assay methods for active agents, including
      chemotherapeutic agents, using cohesive multicellular particulates
IN   **Kornblith, Paul L.**
PA   Precision Therapeutics, Inc., USA
SO   PCT Int. Appl., 16 pp.
      CODEN: PIXXD2
DT   Patent
LA   English
IC   ICM A01N001-02
      ICS C12N001-02; C12N005-00; C12Q001-02; C12Q001-18; C12Q001-24
CC   1-1 (Pharmacology)
FAN.CNT 3
      PATENT NO.   KIND DATE   APPLICATION NO.   DATE
      ---------------   ---- --------   ---------------   --------
PI   WO 9802038   A1   19980122   WO 1997-US11595 19970710
          W: AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ,
              CZ, DE, DE, DK, DK, EE, EE, ES, FI, FI, GB, GE, GH, HU, IL, IS,
              JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK,
              MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL,
              TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD,
              RU, TJ, TM
          RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR,
              GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA,
              GN, ML, MR, NE, SN, TD, TG
      US 5728541   A   19980317   US 1996-679056   19960712
      CA 2259984   AA   19980122   CA 1997-2259984 19970710
      AU 9736493   A1   19980209   AU 1997-36493   19970710
      AU 712302   B2   19991104
      EP 912085   A1   19990506   EP 1997-933267   19970710
          R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
              IE, FI
      BR 9710348   A   20000111   BR 1997-10348   19970710
      CN 1275885   A   20001206   CN 1997-196143   19970710
      IL 127964   A1   20010724   IL 1997-127964   19970710
      JP 2002501609   T2   20020115   JP 1998-506069   19970710
      NZ 333830   A   20021220   NZ 1997-333830   19970710
      US 2002192638   A1   20021219   US 2002-205887   20020726
PRAI US 1996-679056   A   19960712
      WO 1997-US11595   W   19970710
      US 1998-40161   A1   19980317
AB   An improved system is disclosed for screening a multiple of candidate
      therapeutic or chemotherapeutic agents for efficacy as to a specific
      patient, in which a tissue sample from the patient is harvested, cultured
      and sep. exposed to a plurality of treatments and/or therapeutic agents
      for the purpose of objectively identifying the best treatment or agent for
      the particular patient. Specific method innovations such as tissue sample
      prepn. techniques render this method practically as well as theor. useful.
      One particularly important tissue sample prepn. technique is the initial
      prepn. of cohesive multicellular particulates of the tissue sample, rather
      than enzymically dissocd. cell suspensions or prepns., for initial tissue
      culture monolayer prepn. By subjecting uniform samples of cells to a wide

variety of active agents (and concns. thereof), the most promising agent
and concn. for treatment of a particular patient can be detd.
ST   drug screening tissue culture; chemotherapeutic screening tissue culture
IT   Apparatus
        (Terasaki dispenser; precise efficacy assay methods for active agents,
        including chemotherapeutic agents, using cohesive multicellular
        particulates)
IT   Radiotherapy
        (agents for; precise efficacy assay methods for active agents,
        including chemotherapeutic agents, using cohesive multicellular
        particulates)
IT   Body fluid
        (effusion; precise efficacy assay methods for active agents, including
        chemotherapeutic agents, using cohesive multicellular particulates)
IT   **Animal cell**
        Animal tissue
        Animal tissue culture
        Antitumor agents
        Ascites
        Chemotherapy
        Cytotoxic agents
        Drug screening
        Drugs
        Immunotherapy
        Radioprotectants
        Radiosensitizers, biological
        Wound healing promoters
        (precise efficacy assay methods for active agents, including
        chemotherapeutic agents, using cohesive multicellular particulates)
IT   Neoplasm
        (tissue; precise efficacy assay methods for active agents, including
        chemotherapeutic agents, using cohesive multicellular particulates)
RE.CNT 5   THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD
RE
(1) Morgan; US 5270172 A 1993
(2) Naughton; US 5443950 A 1995 HCAPLUS
(3) Rotman; US 4937187 A 1990 HCAPLUS
(4) Stampfer; US 4423145 A 1983
(5) Yen-Maguire; US 5242806 A 1993

L89 ANSWER 5 OF 5 HCAPLUS COPYRIGHT 2003 ACS
AN   1985:3969 HCAPLUS
DN   102:3969
TI   Correlation of experimental and clinical studies of metabolism by PET
     scanning
AU   **Kornblith, Paul L.**; Cummins, C. J.; Smith, Barry H.; Brooks,
     Rodney A.; Patronas, Nicholas J.; Di Chiro, Giovanni
CS   Dep. Health and Human Serv., Natl. Inst. Neurol. Communicative Disorders
     and Stroke, Bethesda, MD, USA
SO   Progress in Experimental Tumor Research (1984), 27(Brain Tumor Biol.),
     170-8
     CODEN: PEXTAR; ISSN: 0079-6263
DT   Journal
LA   English
CC   14-1 (Mammalian Pathological Biochemistry)
     Section cross-reference(s): 9
AB   There was a high pos. correlation between glucose uptake by human glioma
     measured in situ by the 18F-labeled 2-deoxyglucose positron emission
     tomog. (qFDG-PET) procedure and the glucose uptake measured biochem. in
     tissue culture lines obsd. from the same tumor. Glucose metab. was
     related to tumor grade, with grade-IV gliomas showing a mean rate of
     glucose metab. (LCMRGlu) 1.6-fold greater than that of contralateral
     normal brain. Grade-III gliomas had a near-normal LCMRGlu, and low-grade

gliomas had a somewhat depressed metabolic rate. Both hexokinase and
phosphofructokinase (glycolysis-regulating enzymes) were elevated and
glucose-6-phosphate dehydrogenase (a phosphogluconate pathway enzyme) was
decreased, in high-grade gliomas. These enzyme alterations were
consistent with the increased LCMRGlu obsd. in high-grade gliomas. The
pathophysiol. significance of the data and applications of the FDG-PET and
tissue culture methods to tumor grading, follow-up, and metab. are
discussed.
```
ST    glioma metab positron emission tomog; glucose metab glioma methodol
IT    Glycolysis  .
         (by glioma of human, positron emission tomog. vs. tissue culture in
         study of)
IT    Animal tissue culture
         (fro glucose metab. by glioma of human, positron emission tomog. in
         relation to)
IT    Neoplasm, metabolism
         (glucose metab. by, of brain of human, positron emission tomog. vs.
         tissue culture in study of)
IT    Enzymes
      RL: BIOL (Biological study)
         (glycolytic, of glioma of human, tumor grade in relation to)
IT    Radiography
         (laminog., positron, computerized, for glucose metab. by glioma of
         human, tissue culture in relation to)
IT    Neuroglia
         (neoplasm, glucose metab. by human, positron emission tomog. vs. tissue
         culture in study of)
IT    50-99-7, biological studies
      RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL
      (Biological study); PROC (Process)
         (metab. of, by glioma of human, positron emission tomog. vs. tissue
         culture in study of)
IT    9001-40-5    9001-51-8    9001-80-3
      RL: BIOL (Biological study)
         (of glioma of human, glucose metab. and tumor grade in relation to)


=> d tot


L107 ANSWER 1 OF 5  HCAPLUS   COPYRIGHT 2003 ACS
AN    2002:575663   HCAPLUS
DN    137:106071
TI    Immortalized human keratinocyte cell line
IN    Allen-Hoffmann, B. Lynn
PA    USA
SO    U.S. Pat. Appl. Publ., 19 pp., Cont.-in-part of U.S. Ser. No. 769,124.
      CODEN: USXXCO
DT    Patent
LA    English
IC    ICM  C12Q001-70
      ICS  C12Q001-68; G01N033-567; C12N007-00; C12N007-01; C12N005-00;
         C12N005-02; C12N005-06; C12N005-08
NCL   435325000
CC    9-11 (Biochemical Methods)
      Section cross-reference(s): 1, 62, 63
FAN.CNT 4
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	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE	
PI	US 2002102726	A1	20020801	US 2001-945136	20010831	<--
	US 6514711	B2	20030204			
	US 5989837	A	19991123	US 1998-114557	19980713	<--
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       US 2001023061      A1    20010920      US 2001-769124    20010124
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PRAI  US 1998-114557      A1    19980713   <--
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       US 2001-769124     A2    20010124
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AB   The invention concerns a method for testing the effects of various factors
       on human skin equiv. is disclosed. The method involves providing a human
       stratified squamous epithelial cell culture of an immortalized human
       keratinocyte cell line that forms a reconstructed epidermis, exposing the
       reconstructed epidermis to a factor and evaluating the effect of the
       factor on the reconstructed epidermis. A method for selecting preventive
       or therapeutic agents for skin damages caused by a factor using the same
       human stratified squamous epithelial cell culture system is also
       disclosed.

ST   immortalized keratinocyte skin culture drug screening green fluorescent
       protein

IT   **Animal cell line**
           (ATCC CRL-12191; immortalized human keratinocyte cell line)
IT   **Animal cell line**
           (BC-1-Ep/SL; immortalized human keratinocyte cell line)
IT   Pressure
           (air; immortalized human keratinocyte cell line)
IT   Cosmetics
           (creams; immortalized human keratinocyte cell line)
IT   **Animal cell**
           (damage to; immortalized human keratinocyte cell line)
IT   Skin
           (epidermis; immortalized human keratinocyte cell line)
IT   Gene, animal
       RL: BSU (Biological study, unclassified); PRP (Properties); BIOL
       (Biological study)
           (exogenous; immortalized human keratinocyte cell line)
IT   Emulsions
       Liquids
           (form; immortalized human keratinocyte cell line)
IT   Proteins
       RL: BSU (Biological study, unclassified); PRP (Properties); BIOL
       (Biological study)
           (green fluorescent; immortalized human keratinocyte cell line)
IT   Transformation, neoplastic
           (immortalization; immortalized human keratinocyte cell line)
IT   **Animal tissue culture**
       Cell death
       Cell differentiation
       Cell proliferation
       Chromosome
       Climate
       Combinatorial library
       Cosmetics
       DNA sequence analysis
       Detergents
         Drug screening
       Drugs
         Fibroblast
       Genetic methods
       Histochemistry
       Human
       Microscopy
       Skin
       Surfactants
       Toxicity
       UV radiation
           (immortalized human keratinocyte cell line)

IT   Reporter gene
      RL: BSU (Biological study, unclassified); PRP (Properties); BIOL
      (Biological study)
          (immortalized human keratinocyte cell line)
IT   Collagens, uses
      RL: NUU (Other use, unclassified); USES (Uses)
          (immortalized human keratinocyte cell line)
IT   Bases, biological studies
      RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
          (immortalized human keratinocyte cell line)
IT   Chemicals
          (industrial; immortalized human keratinocyte cell line)
IT   Acids, biological studies
      RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
          (inorg.; immortalized human keratinocyte cell line)
IT   Skin
          (keratinocyte; immortalized human keratinocyte cell line)
IT   Bases, biological studies
      RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
          (org.; immortalized human keratinocyte cell line)
IT   **Epithelium**
          (stratified squamous; immortalized human keratinocyte cell line)
IT   **Animal cell**
          (survival of; immortalized human keratinocyte cell line)
IT   1746-01-6, TCDD
      RL: BUU (Biological use, unclassified); BIOL (Biological study); USES
      (Uses)
          (immortalized human keratinocyte cell line)

L107 ANSWER 2 OF 5 HCAPLUS COPYRIGHT 2003 ACS
AN   2001:703739 HCAPLUS
DN·   135:269653
TI   Use of multipotent neural stem cells and their progeny for the screening
      of drugs and other biological agents
IN   Weiss, Samuel; Reynolds, Brent; Hammang, Joseph P.; Baetge, E. Edward
PA   Neurospheres Holdings, Ltd., Can.
SO   U.S., 42 pp., Cont.-in-part of U.S. Ser. No. 385,404, abandoned.
      CODEN: USXXAM
DT   Patent
LA   English
IC   G01N033-554; C12N005-00
NCL   435007210
CC   **9-11 (Biochemical Methods)**
      Section cross-reference(s): 1, 13, 14
FAN.CNT 8

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE	
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AU 665012	B2	19951214			
EP 594669	A1	19940504	EP 1992-914286	19920707	<--

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WO 9409119	A1	19940428	WO 1993-CA428	19931015	<--

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          SE, SK, UA, VN
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AU 9351474           A1    19940509       AU 1993-51474     19931015 <--
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EP 664832            B1    20020724
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JP 08502172          T2    19960312       JP 1993-509473    19931015 <--
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          SE, SK, UA, VN
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AU 9453676           A1    19940524       AU 1994-53676     19931027 <--
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CA 2175992           AA    19950518       CA 1994-2175992   19941108 <--
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EP 728194            B1    20030108
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NO 9502985           A     19950727       NO 1995-2985      19950727 <--
CN 1161744           A     19971008       CN 1995-195867    19950922 <--
EP 1130394           A1    20010905       EP 2001-101622    19950922 <--
      R:  AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE
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AU 9749241           A1    19980312
PRAI US 1991-726812      B1    19910708   <--
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AB   A culture method for detg. the effect of a biol. agent on multipotent
     neural stem cell progeny is provided. In the presence of growth factors,
     multipotent neural stem cells are induced to proliferate in culture. The
     multipotent neural stem cells may be obtained from normal neural tissue or
     from a donor afflicted with a disease such as Alzheimer's Disease,
     Parkinson's Disease or Down's Syndrome. At various stages in the
     differentiation process of the multipotent neural stem cell progeny, the
     effects of a biol. agent, such as a virus, protein, peptide, amino acid,
     lipid, carbohydrate, nucleic acid or a drug or pro-drug on cell activity
     are detd. Addnl., a method of screening the effects of biol. agents on a
     clonal population of neural cells is provided. The technol. provides an
     efficient method for the generation of large nos. of pre- and post-natal
     neural cells under controlled, defined conditions. The disclosed cultures
     provide an optimal source of normal and diseased neural cells at various
     developmental stages, which can be screened for potential side effects in
     addn. to testing the action and efficacy of different biol. agents.
ST   stem cell neuron culture drug screening differentiation proliferation
     factor
IT   Gene, animal
     RL: BSU (Biological study, unclassified); BIOL (Biological study)
        (NGF; use of multipotent neural stem cells and progeny for screening of
        drugs and other biol. agents)
IT   Disease models
        (Parkinson's; use of multipotent neural stem cells and progeny for
        screening of drugs and other biol. agents)
IT   Neurotrophic factor receptors
     RL: BSU (Biological study, unclassified); BIOL (Biological study)
        (TrkB; use of multipotent neural stem cells and progeny for screening
        of drugs and other biol. agents)
IT   Gene, microbial
     RL: BSU (Biological study, unclassified); BIOL (Biological study)
        (b-galactosidase; use of multipotent neural stem cells and progeny for
        screening of drugs and other biol. agents)
IT   Gene, animal
     RL: BSU (Biological study, unclassified); BIOL (Biological study)
        (bcl-2; use of multipotent neural stem cells and progeny for screening
        of drugs and other biol. agents)
IT   Neurotrophic factors
     RL: BAC (Biological activity or effector, except adverse); BSU (Biological
     study, unclassified); BIOL (Biological study)
        (brain-derived; use of multipotent neural stem cells and progeny for
        screening of drugs and other biol. agents)
IT   Transcription factors
     RL: BAC (Biological activity or effector, except adverse); BSU (Biological
     study, unclassified); BIOL (Biological study)
        (c-fos, pathway inhibitors; use of multipotent neural stem cells and
        progeny for screening of drugs and other biol. agents)
IT   Embryo, animal
        (fetus; use of multipotent neural stem cells and progeny for screening
        of drugs and other biol. agents)

IT Neurotrophic factors
    RL: BAC (Biological activity or effector, except adverse); BSU (Biological
    study, unclassified); BIOL (Biological study)
        (glial-derived; use of multipotent neural stem cells and progeny for
        screening of drugs and other biol. agents)
IT Immunoassay
        (immunocytochem.; use of multipotent neural stem cells and progeny for
        screening of drugs and other biol. agents)
IT Chemokines
    RL: BAC (Biological activity or effector, except adverse); BSU (Biological
    study, unclassified); BIOL (Biological study)
        (macrophage inflammatory protein; use of multipotent neural stem cells
        and progeny for screening of drugs and other biol. agents)
IT GAP-43 (protein)
    RL: BSU (Biological study, unclassified); BIOL (Biological study)
        (membrane phosphoprotein; use of multipotent neural stem cells and
        progeny for screening of drugs and other biol. agents)
IT Transplant and Transplantation
        (neural stem cell; use of multipotent neural stem cells and progeny for
        screening of drugs and other biol. agents)
IT Nerve
        (neuroblast, neurosphere; use of multipotent neural stem cells and
        progeny for screening of drugs and other biol. agents)
IT Nerve
        (neuron; use of multipotent neural stem cells and progeny for screening
        of drugs and other biol. agents)
IT Cell
        (stem, neural; use of multipotent neural stem cells and progeny for
        screening of drugs and other biol. agents)
IT Cytotoxic agents
        (tyrphostins, interfere with c-fos path activation; use of multipotent
        neural stem cells and progeny for screening of drugs and other biol.
        agents)
IT Alzheimer's disease
    Animal tissue culture
    Astrocyte
    Cell differentiation
    Cell proliferation
    Down's syndrome
    Drug screening
    Genetic methods
    Mammal (Mammalia)
    Neuroglia
    Parkinson's disease
    Virus
        (use of multipotent neural stem cells and progeny for screening of
        drugs and other biol. agents)
IT Amino acids, biological studies
    Carbohydrates, biological studies
    Ciliary neurotrophic factor
    Hormones, animal, biological studies
    Lipids, biological studies
    Macrophage inflammatory protein 1.alpha.
    Macrophage inflammatory protein 1.beta.
    Macrophage inflammatory protein 2
    Nucleic acids
    Peptides, biological studies
    Platelet-derived growth factors
    Proteins, general, biological studies
    RL: BAC (Biological activity or effector, except adverse); BSU (Biological
    study, unclassified); BIOL (Biological study)
        (use of multipotent neural stem cells and progeny for screening of
        drugs and other biol. agents)

IT   **Myelin**
      RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL
      (Biological study); PROC (Process)
          (use of multipotent neural stem cells and progeny for screening of
          drugs and other biol. agents)
IT   **Transforming growth factors**
      RL: BAC (Biological activity or effector, except adverse); BSU (Biological
      study, unclassified); BIOL (Biological study)
          (.**alpha**.-; use of multipotent neural stem cells and progeny
          for screening of drugs and other biol. agents)
IT   **Transforming growth factors**
      RL: BAC (Biological activity or effector, except adverse); BSU (Biological
      study, unclassified); BIOL (Biological study)
          (.**beta**.-; use of multipotent neural stem cells and progeny
          for screening of drugs and other biol. agents)
IT   62031-54-3, FGF
      RL: BAC (Biological activity or effector, except adverse); BSU (Biological
      study, unclassified); BIOL (Biological study)
          (b; use of multipotent neural stem cells and progeny for screening of
          drugs and other biol. agents)
IT   80449-02-1, Tyrosine kinase
      RL: BAC (Biological activity or effector, except adverse); BSU (Biological
      study, unclassified); BIOL (Biological study)
          (inhibitors; use of multipotent neural stem cells and progeny for
          screening of drugs and other biol. agents)
IT   179047-86-0   179047-88-2   179047-90-6   179047-91-7   222586-28-9, 1:
      PN: US6294346 SEQID: 1 unclaimed DNA   222586-30-3, 3: PN: US6294346
      SEQID: 3 unclaimed DNA   222586-32-5, 5: PN: US6294346 SEQID: 5 unclaimed
      DNA   222586-34-7, 7: PN: US6294346 SEQID: 7 unclaimed DNA
      RL: PRP (Properties)
          (unclaimed nucleotide sequence; use of multipotent neural stem cells
          and their progeny for the screening of drugs and other biol. agents)
IT   9050-30-0, Heparan sulfate   9061-61-4, NGF   16561-29-8, Phorbol
      12-myristate 13-acetate   24305-27-9, TRH   62229-50-9, EGF   62229-50-9D,
      EGF, liganded derivs.   62996-74-1, Staurosporine   67763-96-6, IGF-1
      106096-92-8, FGF-1   106096-93-9, FGF-2   114949-22-3, Activin
      120685-11-2, CGP-41251
      RL: BAC (Biological activity or effector, except adverse); BSU (Biological
      study, unclassified); BIOL (Biological study)
          (use of multipotent neural stem cells and progeny for screening of
          drugs and other biol. agents)
RE.CNT 121   THERE ARE 121 CITED REFERENCES AVAILABLE FOR THIS RECORD
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L107 ANSWER 3 OF 5 HCAPLUS COPYRIGHT 2003 ACS
AN   2001:464263 HCAPLUS
DN   135:58181
TI   Metastasis models using green fluorescent protein (gfp) as a marker
IN   Tan, Yuying; Chishima, Takashi
PA   AntiCancer, Inc., USA
SO   U.S., 11 pp., Cont.-in-part of U.S. Ser. No. 67,734.
      CODEN: USXXAM
DT   Patent
LA   English
IC   ICM A61K035-00
      ICS A61K048-00; C12N015-63; C12N015-85
NCL   424093210
CC   **9-16 (Biochemical Methods)**
      Section cross-reference(s): 1, 14

FAN.CNT 5

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PI	US 6251384	B1	20010626	US 1999-226856	19990107	<--
	US 6232523	B1	20010515	US 1997-848539	19970428	<--
	US 6235967	B1	20010522	US 1998-49544	19980327	<--
	US 6235968	B1	20010522	US 1998-67734	19980428	<--
	CA 2358439	AA	20000713	CA 2000-2358439	20000107	
	WO 2000040274	A1	20000713	WO 2000-US243	20000107	

       W: AU, CA, JP

      RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL,
         PT, SE

| | EP 1156833 | A1 | 20011128 | EP 2000-902334 | 20000107 | |

      R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
        IE, FI

	JP 2002534398	T2	20021015	JP 2000-592028	20000107	
	US 2002026649	A1	20020228	US 2001-870268	20010529	<--
PRAI	US 1997-848539	A2	19970428	<--		
	US 1998-49544	A2	19980327	<--		
	US 1998-67734	A2	19980428	<--		
	US 1999-226856	A	19990107			
	WO 2000-US243	W	20000107			

AB   A method to follow the progression of metastasis of a primary tumor, which
    method comprises removing fresh organ tissues from a vertebrate subject
    which has been modified to contain tumor cells that express GFP and
    observing the excised tissues for the presence of fluorescence is
    disclosed. The fluorescence can also be monitored by observing the
    tissues in situ. Vertebrate subjects which contain GFP producing tumors
    are useful models to study the mechanism of metastasis, as well as to
    evaluate candidate protocols and drugs. In addn., subjects already
    harboring tumors can be treated so as to modify the endogenous tumors to
    contain GFP. This permits clin. applications. Finally, by injecting a
    contrast dye into a subject harboring a GFP-labeled tumor, angiogenesis in
    the tumor can be obsd. directly.

ST   metastasis model green fluorescence protein marker

IT   **Animal cell line**
      (CHO-K1; metastasis models using green fluorescent protein as a marker)

IT   Dyes
      (Contrast; metastasis models using green fluorescent protein as a
      marker)

IT   Gene
      (expression; metastasis models using green fluorescent protein as a
      marker)

IT   Imaging
      (fluorescent, optical tumor; metastasis models using green fluorescent
      protein as a marker)

IT   Proteins, specific or class
    RL: BUU (Biological use, unclassified); BIOL (Biological study); USES
    (Uses)
      (green fluorescent; metastasis models using green fluorescent protein
      as a marker)

IT   Angiogenesis
      Animal cell
      Animal cell line
      Animal tissue
    Disease models
      Drug screening
    Fluorescence
    Lung, neoplasm
    Mammal (Mammalia)
    Microscopy
    Mouse
    Organ, animal

Retroviral vectors
   Tumor markers
Vertebrate (Vertebrata)
Virus vectors
     (metastasis models using green fluorescent protein as a marker)
IT Neoplasm
     (metastasis; metastasis models using green fluorescent protein as a
     marker)
IT Surgery
     (orthotopic implantation; metastasis models using green fluorescent
     protein as a marker)
RE.CNT 65   THERE ARE 65 CITED REFERENCES AVAILABLE FOR THIS RECORD
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L107 ANSWER 4 OF 5  HCAPLUS  COPYRIGHT 2003 ACS
AN   1999:748234  HCAPLUS
DN   131:319888
TI   Immortalized human keratinocyte cell line
IN   Allen-Hoffmann, B. Lynn; Schlosser, Sandra J.; Pickart, Michael A.
PA   Wisconsin Alumni Research Foundation, USA
SO   U.S., 19 pp.
     CODEN: USXXAM
DT   Patent
LA   English
IC   ICM  G01N033-567
     ICS  C12N005-08
NCL  435007210
CC   9-11 (Biochemical Methods)
     Section cross-reference(s): 1, 14
FAN.CNT 4
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PI   US 5989837         A     19991123      US 1998-114557    19980713 <--
     US 6214567         B1    20010410      US 1999-277295    19990326 <--
     CA 2336717         AA    20000120      CA 1999-2336717   19990712 <--
     WO 2000003244      A2    20000120      WO 1999-US15671   19990712 <--
     WO 2000003244      A3    20010426
         W:  AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ,
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             JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK,
             MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ,
             TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD,
             RU, TJ, TM
         RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK,
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             CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
     AU 9949850         A1    20000201      AU 1999-49850     19990712 <--
     BR 9912047         A     20010403      BR 1999-12047     19990712 <--
     EP 1117423         A2    20010725      EP 1999-933896    19990712 <--
         R:  AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
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     JP 2002520594      T2    20020709      JP 2000-559428    19990712 <--
     NZ 509184          A     20030131      NZ 1999-509184    19990712 <--
     US 2002102726      A1    20020801      US 2001-945136    20010831 <--
     US 6514711         B2    20030204
     US 2002192196      A1    20021219      US 2002-131977    20020424 <--
PRAI US 1998-114557     A1    19980713  <--
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US 1999-277295    A1   19990326
WO 1999-US15671   W    19990712
US 2001-769124    A2   20010124
US 2001-286169P   P    20010424
US 2001-844194    A2   20010427
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AB A spontaneously immortalized human keratinocyte cell line is disclosed. In a preferred embodiment, this cell line is ATCC 12191. In another embodiment of the invention, a method of assaying the effect of a test tumor cell modulation agent is disclosed. The method comprises the steps of obtaining a human stratified squamous epithelial cell culture, wherein the culture comprises human malignant squamous epithelial cells and spontaneously immortalized human keratinocytes, wherein the culture forms a reconstituted epidermis. One then treats the epidermis with a test tumor cell modulation agent and evaluates the growth of the malignant cells within the epidermis.

ST immortalized keratinocyte cell line

IT **Animal cell line**
    (EC; immortalized human keratinocyte cell line)

IT Gene
RL: BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)
    (Heterologous; immortalized human keratinocyte cell line)

IT Films
    (bio-; immortalized human keratinocyte cell line)

IT Skin
    (epidermis; immortalized human keratinocyte cell line)

IT Proteins, specific or class
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
    (green fluorescent; immortalized human keratinocyte cell line)

IT Transformation, neoplastic
    (immortalization; immortalized human keratinocyte cell line)

IT **Animal cell line**
    Animal tissue culture
    Drug screening
  Growth, animal
  Toxicity
  Transformation, genetic
    (immortalized human keratinocyte cell line)

IT **Genetic markers**
RL: BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)
    (immortalized human keratinocyte cell line)

IT Skin
    (keratinocyte; immortalized human keratinocyte cell line)

IT **Animal tissue culture**
    (monolayer; immortalized human keratinocyte cell line)

IT **Animal tissue culture**
    (organotypic; immortalized human keratinocyte cell line)

IT **Epithelium**
    (stratified squamous; immortalized human keratinocyte cell line)

RE.CNT 9   THERE ARE 9 CITED REFERENCES AVAILABLE FOR THIS RECORD

RE
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L107 ANSWER 5 OF 5 HCAPLUS COPYRIGHT 2003 ACS
AN   1999:450863 HCAPLUS
DN   131:99526
TI   Immortalized human bone marrow endothelial cell line and their adhesion to
      cancer cells and uses in treatment of metastasis
IN   Pienta, Kenneth J.
PA   The Regents of the University of Michigan, USA
SO   U.S., 13 pp.
      CODEN: USXXAM
DT   Patent
LA   English
IC   ICM G01N033-53   ·
      ICS C12N005-00
NCL   435007230
CC   **9-11 (Biochemical Methods)**
      Section cross-reference(s): 13, 63
FAN.CNT 1
      PATENT NO.   KIND DATE   APPLICATION NO. DATE
      ---------------   ---- --------   --------------- --------
PI   US 5925531   A   19990720   US 1997-956844   19971023 <--
PRAI US 1997-956844   19971023   <--
AB   The present invention provides immortalized human bone marrow endothelial
      cells which are useful for the study of tumor metastasis. Primary bone
      marrow endothelial cells from a 25-yr-old Caucasian man were immortalized
      with SV40 large T antigen to create the HBME-1 cell line. Karyotyping
      revealed a heterogeneous karyotype with both diploid and hyper-tetraploid
      populations of cells. The cells adhere to cancer cells, are easily
      harvested from tissue culture by trypsinization, and grow well in std.
      DMEM supplemented with 10% FBS. In particular, the human bone marrow
      endothelial cell lines provided by the invention provide an in vitro model
      system for screening compds. for the ability to reduce, prevent, or
      inhibit the metastasis of cancer cells to bone tissue.
ST   bone marrow epithelium cell immortalization; cancer cell adhesion bone
      marrow epithelium cell; metastasis bone marrow epithelium cell
IT   **Animal cell line**
          (HBME-1; immortalized human bone marrow endothelial cell line and their
          adhesion to cancer cells and uses in treatment of metastasis)
IT   Intestine, neoplasm
          (colon; immortalized human bone marrow endothelial cell line and their
          adhesion to cancer cells and uses in treatment of metastasis)
IT   Agglutinins and Lectins
      RL: BAC (Biological activity or effector, except adverse); BSU (Biological
      study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES
      (Uses)
          (galactose-binding, galectin, screening compds. for modulating binding
          of epithelial and cancer cells; immortalized human bone marrow
          endothelial cell line and their adhesion to cancer cells and uses in
          treatment of metastasis)
IT   Transformation, neoplastic
          (immortalization; immortalized human bone marrow endothelial cell line
          and their adhesion to cancer cells and uses in treatment of metastasis)
IT   **Bone marrow**
      Cell adhesion
      Disease models
        Drug screening
      Neoplasm
          (immortalized human bone marrow endothelial cell line and their
          adhesion to cancer cells and uses in treatment of metastasis)
IT   Antigens
      RL: BUU (Biological use, unclassified); BIOL (Biological study); USES

(Uses)
    (large T, treatment with SV40 large T antigen for cell line prepn.;
    immortalized human bone marrow endothelial cell line and their adhesion
    to cancer cells and uses in treatment of metastasis)
IT **Antitumor agents**
    Neoplasm
       (metastasis; immortalized human bone marrow endothelial cell line and
       their adhesion to cancer cells and uses in treatment of metastasis)
IT Mammary gland
    Prostate gland
       (neoplasm; immortalized human bone marrow endothelial cell line and
       their adhesion to cancer cells and uses in treatment of metastasis)
IT RGD peptides
    RL: BAC (Biological activity or effector, except adverse); BSU (Biological
    study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES
    (Uses)
       (screening compds. for modulating binding of epithelial and cancer
       cells; immortalized human bone marrow endothelial cell line and their
       adhesion to cancer cells and uses in treatment of metastasis)
IT 99896-85-2
    RL: BAC (Biological activity or effector, except adverse); BSU (Biological
    study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES
    (Uses)
       (screening compds. for modulating binding of epithelial and cancer
       cells; immortalized human bone marrow endothelial cell line and their
       adhesion to cancer cells and uses in treatment of metastasis)
RE.CNT 33   THERE ARE 33 CITED REFERENCES AVAILABLE FOR THIS RECORD

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              E E3+ALL
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L14        3747 S TRANSFORMING GROWTH FACTOR ALPHA
              E TRANSFORMING GROWTH FACTOR/CT
              E E4+ALL
              E E2+ALL
L15        4433 S E5,E6,E4
L16        6164 S TGF(S)ALPHA
L17         184 S TRANSFORMING(L)GROWTH(L)SUBSTANCE(L)ALPHA
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L19       19473 S TRANSFORMING GROWTH FACTOR BETA
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              E TRANSFORMING GROWTH FACTOR .BETA./CT
              E E3+ALL
              E E2+ALL
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L29       35914 S E4,E3+NT


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L32         117 S UROKINASE(S)PLASMINOGEN(S)ACTIVATOR
L33         111 S ALPHA(S)FETOPROTEIN
L34         385 S CARCINOEMBRYONIC(S)ANTIGEN
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L35        303 S TRANSFORMING(S)GROWTH(S)FACTOR(S)ALPHA
L36       1147 S TRANSFORMING(S)GROWTH(S)FACTOR(S)BETA
L37       1268 S MAJOR(S)HISTOCOMPATIBILITY(S)COMPLEX

      FILE 'HCAPLUS' ENTERED AT 09:09:59 ON 02 APR 2003
L38      13743 S L30 OR L31 OR L32 OR L33 OR L34 OR L35 OR L36 OR L37
L39      91700 S L1-L29,L38
             E TUMOR ANTIGEN/CT
             E E5+ALL
L40       3998 S E2
L41       6727 S TUMOR ANTIGEN OR TUMOR ASSOC? ANTIGEN
             E CELL MARKER/CT
             E CELLULAR MARKER/CT
L42       2267 S (CELL OR CELLULAR)()MARKER
L43      23225 S SECRET?(S)FACTOR
L44       6971 S SECRET?(S)GROWTH(S)FACTOR
L45        252 S SECRET?(S)GROWTH(S)SUBSTANCE
L46     121483 S L39-L45
             E BIOPSY/CT
             E BIOPSY
             E BIOP
             E BIOPS
L47      24524 S E11-E23
L48          8 S E29-E32
L49       1065 S L46 AND L47,L48
L50       4256 S L46 AND ?MALIGN?
             E CULTURE/CT
L51       8429 S E4-E10
             E E10+ALL
             E E2+ALL
L52       8287 S E2,E1+NT
             E E10+ALL
L53      29944 S E2,E3,E1+NT
             E E9+ALL
L54     903575 S E3,E2+NT
L55      40371 S L46 AND L51-L54
L56       1551 S L49,L50 AND L55
             E DRUG SCREENING/CT
L57        886 S E4,E5
             E E3+ALL
L58      23428 S E2,E1
L59         85 S E7
             E E5+ALL
L60       5879 S E3+NT
L61       3660 S E12+NT OR E14+NT
L62       3193 S E16+NT
L63       1030 S L46 AND L57-L62
L64        502 S L63 AND L55
L65         22 S L64 AND L56
             E NEOPLASM INHIBITOR/CT
             E E4+ALL
             E E1+ALL
L66      76958 S E1
             E E2+ALL
L67      79405 S E3+NT
L68        297 S L66,L67 AND L56
L69         16 S L68 AND L63
             E KORNBLITH P/AU
L70         47 S E3-E7
             E PRECITION/CS,PA
             E PRECISION/CS,PA
L71          3 S (PRECISION(L)THERAP?)/PA,CS
L72          2 S L71 NOT SEMICONDUCTOR
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L73       37370 S L51-L53
L74           5 S L73 AND L70,L72
L75           6 S (BIOCHEM?(L)METHOD?)/SC,SX AND L70,L72
L76           7 S L72,L74,L75
L77           5 S L75 NOT CEREBELLAR/TI
L78          42 S L70 NOT L77
                SEL DN AN 3
L79           1 S L78 AND E1-E3
L80           6 S L77,L79
L81           5 S L80 AND L38-L69
                E TUMOR MARKER/CT
                E E4+ALL
L82       11274 S E2+NT
L83       30681 S E1+NT
L84           1 S L80 AND L82,L83
L85           5 S L81,L84
                E ANIMAL TISSUE/CT
L86      327587 S E3+NT
                E E3+ALL
L87           2 S E3
L88           4 S L70 AND L86,L87
L89           5 S L85,L88


     FILE 'HCAPLUS' ENTERED AT 09:38:30 ON 02 APR 2003
L90       23500 S L57-L59
L91     1177351 S L53,L54,L86
L92        6937 S L90 AND L91
L93        1284 S L92 AND (BIOCHEM?(L)METHOD?)/SC,SX
L94         286 S L93 AND L66,L67
L95         157 S L93 AND L46
L96         141 S L93 AND L82,L83
L97          97 S L94-L96 AND (PY<=1998 OR PRY<=1998 OR AY<=1998)
L98          52 S L97 NOT 3/SC,SX
L99          50 S L98 NOT L89
L100         23 S L99 AND 9/SC
                E EPITHELIUM/CT
                E E3+ALL
L101      14857 S E3+NT
L102         65 S L101 AND L93
L103         16 S L102 AND (PY<=1998 OR PRY<=1998 OR AY<=1998)
L104         14 S L103 NOT L89
L105         36 S L100,L104
L106         23 S L99 NOT L105
L107          5 S (137:106071 or 135:269653 or 135:58181 or 131:319888 or
=> fil medline
FILE 'MEDLINE' ENTERED AT 10:41:56 ON 08 APR 2003

 FILE LAST UPDATED: 6 APR 2003 (20030406/UP).  FILE COVERS 1958 TO DATE.

 On June 9, 2002, MEDLINE was reloaded.  See HELP RLOAD for details.

 MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the
 MeSH 2003 vocabulary.  See http://www.nlm.nih.gov/mesh/summ2003.html
 for a description on changes.

 This file contains CAS Registry Numbers for easy and accurate
 substance identification.

=> d all tot


L84  ANSWER 1 OF 8     MEDLINE
AN   2003098807      MEDLINE
DN   22498380    PubMed ID: 12610871
```

TI Histoculture drug response assay (HDRA) guided induction concurrent
    chemoradiotherapy for mediastinal node-positive non-small cell lung
    cancer.
AU Yoshimasu Tatsuya; Oura Shoji; Hirai Issei; Kokawa Yozo; Sasaki Rie; Honda
    Kentaro; Tanino Hirokazu; Sakurai Teruhisa; Okamura Yoshitaka
CS Dept. of Thoracic and Cardiovascular Surgery, Wakayama Medical University.
SO GAN TO KAGAKU RYOHO [JAPANESE JOURNAL OF CANCER AND CHEMOTHERAPY], (2003
    Feb) 30 (2) 231-5.
    Journal code: 7810034. ISSN: 0385-0684.
CY Japan
DT Journal; Article; (JOURNAL ARTICLE)
LA Japanese
FS Priority Journals
EM 200303
ED Entered STN: 20030304
    Last Updated on STN: 20030311
    Entered Medline: 20030310
AB To improve the response to chemotherapy for non-small cell lung cancer
    (NSCLC), effective drugs should be selected for each patient. In 1994 we
    introduced histoculture drug response assay (HDRA) for NSCLC patients. For
    clinical N2 patients, **biopsy** of mediastinal lymph node is
    performed both for histological diagnosis and for HDRA. Induction
    concurrent chemoradiotherapy is then performed using HDRA positive
    chemotherapy agents. We have treated three patients with this strategy.
    HDRA could be performed using mediastinal lymph node **biopsy**
    specimens. Tumor reduction rates of these patients were 80.4%, 85.3%, and
    57.1%. Their histological responses were Ef.3, Ef.2, and Ef.1b,
    respectively. Complete resection was done in all patients. This strategy
    appeared to be useful in NSCLC patients with mediastinal lymph node
    metastasis.
CT Check Tags: Female; Human; Male
      Aged
      Antineoplastic Combined Chemotherapy Protocols: AD, administration &
    dosage
       *****Antineoplastic Combined Chemotherapy Protocols: TU, therapeutic**
    use
        *****Carcinoma, Non-Small-Cell Lung: DT, drug therapy**
        Carcinoma, Non-Small-Cell Lung: RT, radiotherapy
        Carcinoma, Non-Small-Cell Lung: SU, surgery
      Cisplatin: AD, administration & dosage
      Combined Modality Therapy
        Drug Screening Assays, Antitumor
      English Abstract
      Etoposide: AD, administration & dosage
       *****Lung Neoplasms: DT, drug therapy**
       *****Lung Neoplasms: RT, radiotherapy**
        Lung Neoplasms: SU, surgery
        Lymphatic Metastasis
      Middle Age
      Paclitaxel: AD, administration & dosage
     *Paclitaxel: AA, analogs & derivatives
      Pneumonectomy
        Tumor Cells, Cultured
RN 114977-28-5 (docetaxel); 15663-27-1 (Cisplatin); 33069-62-4 (Paclitaxel);
    33419-42-0 (Etoposide)
CN 0 (Antineoplastic Combined Chemotherapy Protocols); 0 (VP-P protocol)

L84 ANSWER 2 OF 8   MEDLINE
AN **92253979**   MEDLINE
DN **92253979**   PubMed ID: **1579821**
TI [Resistance of cancer cells to antineoplastic agents. Can the efficiency
    of chemotherapy be predicted?].
    Resistance des cellules cancereuses aux agents antineoplasiques. Peut-on

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        predire l'efficacite d'une chimiotherapie?.
AU      Benard J
SO      REVUE DU PRATICIEN, (1992 Feb 1) 42 (3) 318-9.  Ref: 8
        Journal code: 0404334. ISSN: 0035-2640.
CY      France
DT      Editorial
        General Review; (REVIEW)
        (REVIEW, TUTORIAL)
LA      French
FS      Foreign
EM      199206
ED      Entered STN: 19920619
        Last Updated on STN: 19970203
        Entered Medline: 19920611
CT      Check Tags: Human
          *Antineoplastic Agents: PD, pharmacology
         Drug Resistance
           Neoplasms: GE, genetics
          *Tumor Cells, Cultured: DE, drug effects
           Tumor Markers, Biological: GE, genetics
CN      0 (Antineoplastic Agents); 0 (Tumor Markers, Biological)


L84     ANSWER 3 OF 8      MEDLINE
AN      91274143      MEDLINE
DN      91274143    PubMed ID: 2054260
TI      Three-dimensional histoculture: origins and applications in cancer
        research.
AU      Hoffman R M
CS      Laboratory of Cancer Biology, University of California, San Diego, La
        Jolla 92093-0609.
SO      CANCER CELLS, (1991 Mar) 3 (3) 86-92.  Ref: 75
        Journal code: 9000382. ISSN: 1042-2196.
CY      United States
DT      Journal; Article; (JOURNAL ARTICLE)
        General Review; (REVIEW)
        (REVIEW, TUTORIAL)
LA      English
FS      Priority Journals
EM      199108
ED      Entered STN: 19910818
        Last Updated on STN: 19970203
        Entered Medline: 19910801
AB      The ability to grow cells in monolayer culture has afforded investigators
        the opportunity to study many aspects of cancer cell biology under
        carefully controlled conditions. Nonetheless, an important factor that has
        often been overlooked is that cells in this configuration undergo a loss
        of structural integrity that may significantly alter their functional
        properties. Three-dimensional histoculture represents a useful alternative
        approach to monolayer culture because it preserves the native architecture
        of cells while still allowing ease of experimental manipulation. This
        review discusses the origins of three-dimensional cultures, the potential
        application of these cultures to assays of tumor cell metastasis and drug
        sensitivity, and the evidence from gene expression studies that these
        cultures may be more realistic tumor models than cell monolayers.
CT      Check Tags: Animal; Human
          Antigens, Neoplasm: BI, biosynthesis
         Cell Aggregation
         Cell Movement
         Cellulose
         Collagen
          Drug Screening Assays, Antitumor
         Gene Expression Regulation, Neoplastic
           Neoplasm Invasiveness: PA, pathology
```

         Neoplasm Metastasis: PA, pathology
         Organ Culture: IS, instrumentation
         Organ Culture: MT, methods
         Tissue Culture: IS, instrumentation
        *Tissue Culture: MT, methods
         Tumor Cells, Cultured: DE, drug effects
         Tumor Cells, Cultured: IM, immunology
        *Tumor Cells, Cultured: PA, pathology
RN   9004-34-6 (Cellulose); 9007-34-5 (Collagen)
CN   0 (Antigens, Neoplasm)

L84   ANSWER 4 OF 8   MEDLINE
AN   **90141259**   MEDLINE
DN   **90141259**   PubMed ID: **401249**
TI   "Invasiveness" in tissue culture: a technique for study of gliomas.
AU   Scott R M; Liszczak T M; **Kornblith P L**
CS   Department of Neurosurgery, Tufts University School of Medicine, Boston.
SO   SURGICAL FORUM, (1978) 29 531-3.
      Journal code: 0337723. ISSN: 0071-8041.
CY   United States
DT   Journal; Article; (JOURNAL ARTICLE)
LA   English
FS   Priority Journals
EM   199002
ED   Entered STN: 19900328
      Last Updated on STN: 19900328
      Entered Medline: 19900226
CT   Check Tags: Human
        *Glioma: PA, pathology
       Methods
          Neoplasm Invasiveness
          Tissue Culture

L84   ANSWER 5 OF 8   MEDLINE
AN   **88080034**   MEDLINE
DN   **88080034**   PubMed ID: **3121170**
TI   Plasminogen activator and inhibitor activity in human glioma cells and
      modulation by sodium butyrate.
AU   Gross J L; Behrens D L; Mullins D E; **Kornblith P L**; Dexter D L
CS   Medical Products Department, E.I. Du Pont de Nemours & Co., Inc.,
      Wilmington, Delaware 19898.
NC   CA23753 (NCI)
SO   CANCER RESEARCH, (1988 Jan 15) 48 (2) 291-6.
      Journal code: 2984705R. ISSN: 0008-5472.
CY   United States
DT   Journal; Article; (JOURNAL ARTICLE)
LA   English
FS   Priority Journals
EM   198802
ED   Entered STN: 19900305
      Last Updated on STN: 19990129
      Entered Medline: 19880223
AB   The activity of the serine protease plasminogen activator (PA), which
      correlates with tumorigenicity and metastatic capacity, was examined using
      the 125I-labeled fibrin plate assay in cell extracts from four human
      glioma lines as a function of growth in vitro. Cell-associated inhibitory
      activity to plasmin and urokinase-type PA was also measured concurrently.
      The relative PA activities differed markedly among the lines, whereas
      inhibitory activities did not. Two lines, SNB-19 and SNB-75, exhibited
      maximal PA activities (1-6 m Plough units/micrograms protein) as cultures
      approached confluence, whereas two other lines, SNB-56 and SNB-78,
      expressed low PA activity at all times (less than 0.2 m Plough
      units/micrograms protein). The PA of SNB-19 cell extracts was

predominantly urokinase-type PA. In addition to having the highest PA
levels, SNB-19 and SNB-75 were the most clonogenic in soft agar and
tumorigenic in nude mice. In contrast, SNB-56 and SNB-78 were poorly
clonogenic in soft agar and were not tumorigenic in nude mice. Measured
directly, inhibitory activities to plasmin, urokinase-type PA, and
tissue-type PA were detected in SNB-19 (high PA) and SNB-56 (low PA) cell
extracts. However, there were no qualitative or quantitative differences
in inhibitor effects between SNB-19 and SNB-56 suggesting that the
differences in PA activity between these lines resulted from changes in PA
activity and were not due to differential plasminogen activator inhibitor
effects. The ability of the differentiating agent sodium butyrate (NaB) to
modulate total PA activity was also examined. Peak SNB-19 cell PA activity
was decreased in a concentration (Ki, 0.75 mM) and time-dependent manner
by the addition of nontoxic amounts of NaB. The dose-dependent decrease in
PA activity induced by NaB was most likely due to an effect on PA itself,
since the action of inhibitor on urokinase was unchanged in response to
NaB. These results suggest that net cellular PA activity in glioma cells
is a balance between relative PA activity and inhibitor(s) effects and
that this balance can be modulated by sodium butyrate.

CT	Check Tags: Animal; Human; Support, U.S. Gov't, P.H.S.
	*Butyrates: PD, pharmacology
	Butyric Acid
	*Butyric Acids: PD, pharmacology
	Dose-Response Relationship, Drug
	Glioma: AN, analysis
	Glioma: GE, genetics
	Glioma: PA, pathology
	*Glycoproteins: AN, analysis
	Mice
	Mice, Nude
	Neoplasm Transplantation
	*Plasminogen Activators: AN, analysis
	Plasminogen Activators: AI, antagonists & inhibitors
	Plasminogen Inactivators
	Transplantation, Heterologous
	Tumor Cells, Cultured
RN	107-92-6 (Butyric Acid)
CN	0 (Butyrates); 0 (Butyric Acids); 0 (Glycoproteins); 0 (Plasminogen Inactivators); EC 3.4.21.- (Plasminogen Activators)

L84	ANSWER 6 OF 8 MEDLINE
AN	**85296751** MEDLINE
DN	**85296751** PubMed ID: **4033960**
TI	Role of cell and explant culture in the diagnosis and characterization of human pituitary tumours.
AU	Adams E F; Mashiter K
SO	NEUROSURGICAL REVIEW, **(1985)** 8 (3-4) 135-40. Journal code: 7908181. ISSN: 0344-5607.
CY	GERMANY, WEST: Germany, Federal Republic of
DT	Journal; Article; (JOURNAL ARTICLE)
LA	English
FS	Priority Journals
EM	198510
ED	Entered STN: 19900320 Last Updated on STN: 19900320 Entered Medline: 19851022
AB	The pattern of hormone **secretion** by human pituitary tumours in cell or explant culture has been shown to be of value in establishing the nature of the tissue. There was complete agreement between the diagnosis reached by conventional immunocytochemical techniques and by examining the **secretion** of hormones in culture. Culture techniques, however, have some advantages over immunocytochemical analysis. In particular, immunocytochemical techniques can only be used to examine a small,

possibly unrepresentative, portion of the pituitary tissue, whereas the in
vitro culture systems make use of the whole tissue. In addition, in vitro
culture is simpler to employ and can be carried out relatively rapidly.
Cell and explant culture was therefore used to examine the nature of human
pituitary tumours. To determine the incidence of mixed GH-PRL
secreting pituitary tumours in acromegaly, the pattern of hormone
secretion in vitro by 98 somatotrophic tumours was examined.
Thirty-seven per cent were found to be pure somatotrophic tumours and
59.2% **secreted** both GH and PRL, but no other hormone, indicating
that these tumours were of mixed nature. This latter group could be
divided into those removed from patients with hyperprolactinaemia (35.7%
of all tumours) and those from patients with normal pre-operative serum
PRL levels (23.5%). A further small group (3.1%) of tumours
secreted only GH in culture, despite elevated pre-operative serum
PRL levels, ·indicating that the hyperprolactinaemia in these patients was
due to pituitary stalk compression by the somatotrophic tumour, thereby
preventing prolactin release inhibiting **factor** reaching the
lactotrophs and allowing uncontrolled PRL **secretion**.(ABSTRACT
TRUNCATED AT 250 WORDS)

CT	Check Tags: Human; In Vitro
	Gonadotropins: SE, secretion
	*Pituitary Neoplasms: DI, diagnosis
	Pituitary Neoplasms: SE, secretion
	Prolactin: BL, blood
	Prolactin: SE, secretion
	Somatotropin: SE, secretion
	*Tissue Culture
RN	9002-62-4 (Prolactin); 9002-72-6 (Somatotropin)
CN	0 (Gonadotropins)

L84	ANSWER 7 OF 8 MEDLINE
AN	**79044379** MEDLINE
DN	**79044379** PubMed ID: **213226**
TI	Role of tissue culture in prediction of malignancy.
AU	**Kornblith P L**
SO	CLINICAL NEUROSURGERY, (1978) 25 346-76.
	Journal code: 2985141R. ISSN: 0069-4827.
CY	United States
DT	Journal; Article; (JOURNAL ARTICLE)
LA	English
FS	Priority Journals
EM	197901
ED	Entered STN: 19900314
	Last Updated on STN: 19970203
	Entered Medline: 19790126
AB	It is now clear that tissue culture has a role to play in the prediction
	of the degree of malignancy of human brain tumors. The fact that virtually
	all human brain tumors grow at least for some interval in culture allows
	application of tissue culture to the study of all tumors. This almost
	universal culturability of tumors is truly a singular virtue of
	intracranial neoplasms. No other human solid tumor group has been so
	amenable to in vitro growth or study. Up to the present time, despite our
	experience with over 1100 human brain tumor cultures, we have been
	extremely conservative in altering patient management on the basis of in
	vitro data. As is now apparent, the basis for direct input into the
	clinical milieu exists and it is necessary to work on a patient by patient
	basis to see how well the existing criteria can be applied to help guide
	management. Indeed the emphasis given to tissue culture by Rubinstein
	already confirms the current interest in the applicability of tissue
	culture data to neuropathological study of tumors (48). Clearly for
	certain tumors which tend to be benign, tissue culture can serve to alert
	the clinician to the perhaps unexpected malignant potential of the lesion.
	For the malignant tumors, the degree of malignancy, the probable

biological behavior, the role of host defense factors and therapeutic
agents can all be more quantitatively defined for the individual patient
by the detailed study of the patient's cultured cells. With improvement in
the surgical treatment of benign tumors and with better chemotherapeutic
and radiotherapeutic measures for malignant neoplasms this more detailed
and precise characterization of tumor behavior has become increasingly
relevant to the optimization of the clinical management of the brain tumor
patient.

CT   Check Tags: Animal; Human; Support, U.S. Gov't, P.H.S.
       Actins: ME, metabolism
         Astrocytoma: UL, ultrastructure
       Brain: UL, ultrastructure
         Brain Neoplasms: IM, immunology
         Brain Neoplasms: TH, therapy
        ***Brain Neoplasms: UL, ultrastructure**
       Cell Communication
       Cell Survival
       Chromosome Aberrations
         Ependymoma: UL, ultrastructure
       Inclusion Bodies: UL, ultrastructure
       Kinetics
         Medulloblastoma: UL, ultrastructure
         Meningioma: UL, ultrastructure
       Mitosis
         Neoplasm Invasiveness
         Oligodendroglioma: UL, ultrastructure
         Tissue Culture: MT, methods
CN   0 (Actins)

L84   ANSWER 8 OF 8   MEDLINE
AN   **75067689**   MEDLINE
DN   **75067689**   PubMed ID: **4439230**
TI   Improved assay for cytotoxic antiglioma antibody.
AU   Quindlen E A; Dohan F C Jr; **Kornblith P L**
SO   SURGICAL FORUM, (1974) 25 (0) 464-6.
      Journal code: 0337723. ISSN: 0071-8041.
CY   United States
DT   Journal; Article; (JOURNAL ARTICLE)
LA   English
FS   Priority Journals
EM   197503
ED   Entered STN: 19900310
      Last Updated on STN: 19900310
      Entered Medline: 19750318
CT   Check Tags: Animal; Human
      *Antibodies, Neoplasm: AN, analysis
        ***Astrocytoma: IM, immunology**
       Cytotoxicity Tests, Immunologic
       Immunoassay
       Rabbits
CN   .0 (Antibodies, Neoplasm)

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      (FILE 'HOME' ENTERED AT 09:39:27 ON 08 APR 2003)
              SET COST OFF

      FILE 'MEDLINE' ENTERED AT 09:39:55 ON 08 APR 2003
L1   1424959 S C4./CT
L2   169760 S TUMOR CELLS, CULTURED+NT/CT
L3   1192844 S L1/MAJ OR L2/MAJ
              E CULTURE MEDIUM/CT

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                    E  E10+ALL
                    E  E2+ALL
L4         68966  S  E4+NT
L5        636720  S  E15+NT
L6         36910  S  L4/MAJ OR L5/MAJ
L7       1215435  S  L3,L6
L8       1190094  S  L7 AND L1
                    E  DRUG SCREENING/CT
                    E  E4+ALL
L9         10395  S  E17+NT
                    E  E16+ALL
L10        72779  S  E12+NT
L11         6112  S  L8 AND L9
L12         8256  S  L8 AND L10
L13         8256  S  L11,L12
L14         4127  S  L13 AND D22./CT
L15         4127  S  L14 AND L1-L14
L16           96  S  L15 AND BIOPS?
L17            2  S  L16 NOT AB/FA
L18           94  S  L16 NOT L17
L19         2610  S  L15 AND PY<=1996
L20           17  S  L19 AND BIOPSY+NT/CT
L21           62  S  L18 AND L19
L22           64  S  L20,L21
L23            2  S  L22 NOT AB/FA
L24           62  S  L22 NOT L23
L25           51  S  L24/ENG
L26           14  S  L15 AND SECRET?(L)FACTOR
L27           97  S  L15 AND (CELL? OR TUMOR?)(L)MARKER
L28           60  S  L15 AND TUMOR(L)ANTIGEN?
L29            9  S  L15 AND PLASMINOGEN?(L)ACTIVAT?
L30            8  S  L15 AND (URIN? OR UROKINASE)(L)PLASMINOGEN?(L)ACTIVAT?
L31            9  S  L15 AND ALPHA(L)FETOPROTEIN
L32           10  S  L15 AND CARCINOEMBRYONIC(L)ANTIGEN?
L33            7  S  L15 AND TRANSFORM?(L)GROWTH(L)FACTOR(L)ALPHA
L34           12  S  L15 AND TRANSFORM?(L)GROWTH(L)FACTOR(L)BETA
L35            2  S  L15 AND MAJOR(L)HISTOCOMPAT?(L)COMPLEX
L36          203  S  L26-L35
L37           77  S  L36 AND L19
L38            4  S  L37 AND L18,L24
L39           73  S  L37 NOT L38
L40            8  S  L36 AND L18
                    E  KORNBLITH/AU
L41          136  S  E10-E14
L42          123  S  L41 AND L1-L15
                    SEL DN AN 17 19 93 113
L43            4  S  L42 AND E1-E12
L44           13  S  L41 NOT L42
                    E  TUMOR ANTIGENS/CT
                    E  E3+ALL
L45        52163  S  E2+NT
                    E  TUMOR MARKERS/CT
                    E  E7+ALL
L46        72911  S  E8+NT
                    E  E7+ALL
                    E  URINARY PLASMINOGEN/CT
                    E  E4+ALL
L47         7724  S  E34+NT
                    E  TRANSFORMING GROWTH FACTOR/CT
                    E  E4+ALL
L48         2668  S  E57+NT
                    E  TRANSFORMING GROWTH FACTOR BETA/CT
                    E  E3+ALL
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L49      15475 S E81+NT
               E MAJOR HISTOCOMPATIB/CT
               E E5+ALL
L50      15830 S E21+NT
               E ALPHA FETOPROTEIN/CT
               E E4+ALL
L51      10294 S E2+NT
               E CARCINOEMBRYONIC/CT
               E E4+ALL
L52      10097 S E39+NT
               E SECRETED FACTOR/CT
               E SECRET/CT
L53      44516 S SECRET?(S)FACTOR
L54     176472 S L45-L53
L55     108418 S L54 AND PY<=1996
L56      45839 S L55 AND L3
L57       1270 S L55 AND L5/MAJ
L58         23 S L56,L57 AND L4/MAJ
L59         19 S L57 AND L9,L10
L60         19 S L57 AND D22./CT
L61         54 S L58-L60
L62         10 S L61 NOT AB/FA
               E TISSUE CULTURE/CT
               E E3+ALL
L63      68966 S E4+NT
L64       1158 S L63 AND L55
L65          8 S L64 AND L9,L10
L66         10 S L64 AND D22./CT
L67         41 S L64 AND L5/MAJ
L68         98 S L61,L65-L67
L69         20 S L68 NOT AB/FA
               SEL DN AN 2
L70          1 S L69 AND E1-E3
L71         78 S L68 NOT L69
L72          5 S L43,L70
L73       2387 S L57,L64 AND PY<=1996
L74         22 S L73 AND L9
L75         28 S L73 AND D22./CT
L76         46 S L74,L75
               SEL DN AN L16 1
L77          1 S E4-E6
L78          6 S L72,L77 AND L1-L77
L79         48 S L63/MAJ AND L73
L80      10526 S L63/MAJ AND L7
L81       8083 S L80 AND PY<=1996
L82         48 S L81 AND L54
               SEL DN AN 31 40
L83          2 S E7-E12
L84          8 S L78,L83 AND L1-L83


    FILE 'MEDLINE' ENTERED AT 10:41:56 ON 08 APR 2003

    FILE 'WPIX' ENTERED AT 10:42:11 ON 08 APR 2003
L85        568 S C12Q001-24/IC,ICM,ICS
L86       1359 S G01N033-567/IC,ICM,ICS
L87       1911 S L85,L86
L88         47 S A61P035/IC,ICM,ICS,ICA,ICI AND L87
L89        307 S P63?/M0,M1,M2,M3,M4,M5,M6 AND L87
L90        281 S (B14-H? OR C14-H? OR B12-G07 OR C12-G07 OR B12-G05 OR C12-G05
L91        365 S L88-L90
L92        172 S L91 AND (PY<=1996 OR PRY<=1996 OR AY<=1996)
L93         30 S L92 AND A01N/IC,ICM,ICS
L94        158 S L92 AND G01N/IC,ICM,ICS
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L95          92 S L94 AND C12Q/IC,ICM,ICS
L96          17 S L93 AND L95
L97          57 S C12N/IC,ICM,ICS AND L95
L98          13 S L97 AND L96
L99          25 S A61K045/IC,ICM,ICS AND L92
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L2: Entry 2 of 2 File: DWPI Dec 13, 2001

DERWENT-ACC-NO: 2002-097070
DERWENT-WEEK: 200253
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TITLE: Identifying and monitoring progress of malignancy, useful e.g. for selection of therapy, by testing monolayer cultures grown from cohesive multicellular particulates

INVENTOR: KORNBLITH, P L

PATENT-ASSIGNEE:
ASSIGNEE	CODE
KORNBLITH P L	KORNI
PRECISION THERAPEUTICS INC	PRECN

PRIORITY-DATA: 1998US-0189310 (November 10, 1998), 1996US-0679056 (July 12, 1996), 1998US-0039957 (March 16, 1998), 1998US-0095993 (June 11, 1998)

PATENT-FAMILY:
PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
US 20010051353 A1	December 13, 2001		024	C12Q001/02
US 6416967 B2	July 9, 2002		000	C12Q001/02

APPLICATION-DATA:
PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
US20010051353A1	July 12, 1996	1996US-0679056	CIP of
US20010051353A1	March 16, 1998	1998US-0039957	CIP of
US20010051353A1	June 11, 1998	1998US-0095993	CIP of
US20010051353A1	November 10, 1998	1998US-0189310	
US20010051353A1		US 5728541	CIP of
US 6416967B2	July 12, 1996	1996US-0679056	CIP of
US 6416967B2	March 16, 1998	1998US-0039957	CIP of
US 6416967B2	June 11, 1998	1998US-0095993	CIP of
US 6416967B2	November 10, 1998	1998US-0189310	
US 6416967B2		US 5728541	CIP of

INT-CL (IPC): C12 Q 1/02; C12 Q 1/18; C12 Q 1/24

RELATED-ACC-NO: 1998-110245

ABSTRACTED-PUB-NO: US 6416967B
BASIC-ABSTRACT:

NOVELTY - Identifying and monitoring (M1) progress of a patient with a malignancy by:

(i) separating a sample of tumor cells into cohesive multicellular particulates (A);

(ii) growing a tissue culture monolayer from (A) to form a prime culture; and

(iii) monitoring this monolayer over time.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for treating a patient with a malignancy by using (M1) to analyze malignancy-associated markers, and using results of the analysis to determine a treatment regime.

ACTIVITY - Cytostatic. No biodata is given in the source document.

MECHANISM OF ACTION - None given in the source document.

USE - The method is used to identify a malignancy, initially, to analyze cellular and secreted markers (including those indicative of complications, aggressiveness and invasiveness), to study its growth rate and the effects of treatments with various chemotherapeutic agents, including an assessment of side-effects on normal cells, also to select the best therapy in individual cases.

ADVANTAGE - The method can be used throughout the period of the malignancy and is suitable for automation. Growing (A), rather than a suspension culture, overcomes problems of overgrowth by fibroblasts.
ABSTRACTED-PUB-NO:

US20010051353A
EQUIVALENT-ABSTRACTS:

NOVELTY - Identifying and monitoring (M1) progress of a patient with a malignancy by:

(i) separating a sample of tumor cells into cohesive multicellular particulates (A);

(ii) growing a tissue culture monolayer from (A) to form a prime culture; and

(iii) monitoring this monolayer over time.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for treating a patient with a malignancy by using (M1) to analyze malignancy-associated markers, and using results of the analysis to determine a treatment regime.

ACTIVITY - Cytostatic. No biodata is given in the source document.

MECHANISM OF ACTION - None given in the source document.

USE - The method is used to identify a malignancy, initially, to analyze cellular and secreted markers (including those indicative of complications, aggressiveness and invasiveness), to study its growth rate and the effects of treatments with various chemotherapeutic agents, including an assessment of side-effects on normal cells, also to select the best therapy in individual cases.

ADVANTAGE - The method can be used throughout the period of the malignancy and is suitable for automation. Growing (A), rather than a suspension culture, overcomes problems of overgrowth by fibroblasts.

CHOSEN-DRAWING: Dwg.0/9

TITLE-TERMS: IDENTIFY MONITOR PROGRESS MALIGNANT USEFUL SELECT THERAPEUTIC TEST MONOLAYER CULTURE GROW COHERE MULTICELL PARTICLE

DERWENT-CLASS: B04 D16

CPI-CODES: B04-E03G; B04-F02; B04-F02A; B04-K01L; B11-C08E1; B11-C08E3; B12-K04A1; B14-H01; D05-H08; D05-H09;

CHEMICAL-CODES:

Chemical Indexing M1 *01*

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        Fragmentation Code
        M423 M750 M905 N102 N136 N161 Q233
        Specfic Compounds
        A00GTK A00GTA

    Chemical Indexing M1 *02*
        Fragmentation Code
        M423 M750 M781 M905 N102 N136 P831 Q233 Q505
        Specfic Compounds
        A00H3K A00H3A A00H3D

    Chemical Indexing M1 *03*
        Fragmentation Code
        M423 M750 M781 M905 N102 N136 P831 Q233 Q505
        Specfic Compounds
        A012PK A012PA A012PD

    Chemical Indexing M1 *04*
        Fragmentation Code
        M423 M750 M781 M905 N102 N136 P831 Q233 Q505
        Specfic Compounds
        A00NSK A00NSA A00NSD

    Chemical Indexing M6 *05*
        Fragmentation Code
        M905 P633 P831 Q233 Q505 R511 R515 R521 R528 R614
        R637 R639

    SECONDARY-ACC-NO:
    CPI Secondary Accession Numbers: C2002-030166
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L1: Entry 1 of 1   File: DWPI   Dec 19, 2002

DERWENT-ACC-NO: 1998-110245
DERWENT-WEEK: 200303
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TITLE: Assessing chemo-sensitivity of patient cells using mono-layers grown from multicellular particulates - providing samples that relate better to in vivo behaviour, particularly used to select best agents for treating tumours in individual patients

INVENTOR: KORNBLITH, P L

PATENT-ASSIGNEE:

ASSIGNEE	CODE
PRECISION THERAPEUTICS INC	PRECN

PRIORITY-DATA: 1996US-0679056 (July 12, 1996), 1998US-0040161 (March 17, 1998), 2002US-0205887 (July 26, 2002)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
US 20020192638 A1	December 19, 2002		000	C12Q001/00
WO 9802038 A1	January 22, 1998	E	015	A01N001/02
US 5728541 A	March 17, 1998		005	C12Q001/02
AU 9736493 A	February 9, 1998		000	A01N001/02
EP 912085 A1	May 6, 1999	E	000	A01N001/02
AU 712302 B	November 4, 1999		000	A01N001/02
BR 9710348 A	January 11, 2000		000	A01N001/02
CN 1275885 A	December 6, 2000		000	A01N001/02
IL 127964 A	July 24, 2001		000	A01N001/02
JP 2002501609 W	January 15, 2002		014	G01N033/15

DESIGNATED-STATES: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH HU IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG UZ VN YU ZW AT BE CH DE DK EA ES FI FR GB GH GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE

APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
US20020192638A1	July 12, 1996	1996US-0679056	Cont of
US20020192638A1	March 17, 1998	1998US-0040161	Cont of
US20020192638A1	July 26, 2002	2002US-0205887	
US20020192638A1		US 5728541	Cont of
WO 9802038A1	July 10, 1997	1997WO-US11595	
US 5728541A	July 12, 1996	1996US-0679056	
AU 9736493A	July 10, 1997	1997AU-0036493	
AU 9736493A		WO 9802038	Based on
EP 912085A1	July 10, 1997	1997EP-0933267	
EP 912085A1	July 10, 1997	1997WO-US11595	
EP 912085A1		WO 9802038	Based on
AU 712302B	July 10, 1997	1997AU-0036493	
AU 712302B		AU 9736493	Previous Publ.
AU 712302B		WO 9802038	Based on
BR 9710348A	July 10, 1997	1997BR-0010348	
BR 9710348A	July 10, 1997	1997WO-US11595	
BR 9710348A		WO 9802038	Based on
CN 1275885A	July 10, 1997	1997CN-0196143	
IL 127964A	July 10, 1997	1997IL-0127964	
JP2002501609W	July 10, 1997	1997WO-US11595	
JP2002501609W	July 10, 1997	1998JP-0506069	
JP2002501609W		WO 9802038	Based on

INT-CL (IPC): A01 N 1/02; A61 K 45/00; A61 P 17/02; A61 P 31/00; A61 P 35/00; A61 P 37/02; C12 N 1/02; C12 N 5/00; C12 N 5/10; C12 Q 1/00; C12 Q 1/02; C12 Q 1/18; C12 Q 1/24; G01 N 33/15; G01 N 33/48; G01 N 33/50; G01 N 33/567

RELATED-ACC-NO: 2002-097070

ABSTRACTED-PUB-NO: US 5728541A
BASIC-ABSTRACT:

Chemosensitivity of patient cells is assessed by:

(a) separating a specimen of tissue, cell ascites or effusion fluid into multicellular particulates (MP);

(b) growing a tissue culture monolayer from cohesive MP;

(c) inoculating cells from this layer into many separate sites;

(d) treating the sites with test agents, and

(e) assessing chemosensitivity of the treated cells.

USE - The method is used to identify the best treatment agent and concentration for a particular patient, especially for treatment of cancer or other hyper-proliferative diseases such as psoriasis and for wound healing. Formation and blocking of enzymes, neurotransmitters and other biologically active compounds can also be screened for.

ADVANTAGE - By using MP, rather than enzyme-dissociated suspensions, to produce a monolayer, preparation is simplified and a cell culture that retains in vivo reactivity is formed.

Particularly, growth of malignant cells is optimised, without overgrowth of fibroblasts or other cells as often occurs in suspension cultures. The monolayers can be grown in a few weeks, contrast longer times required with single cell progeny by dilution cloning.

ABSTRACTED-PUB-NO:

WO 9802038A
EQUIVALENT-ABSTRACTS:

Chemosensitivity of patient cells is assessed by:

(a) separating a specimen of tissue, cell ascites or effusion fluid into multicellular particulates (MP);

(b) growing a tissue culture monolayer from cohesive MP;

(c) inoculating cells from this layer into many separate sites;

(d) treating the sites with test agents, and

(e) assessing chemosensitivity of the treated cells.

USE - The method is used to identify the best treatment agent and concentration for a particular patient, especially for treatment of cancer or other hyper-proliferative diseases such as psoriasis and for wound healing. Formation and blocking of enzymes, neurotransmitters and other biologically active compounds can also be screened for.

ADVANTAGE - By using MP, rather than enzyme-dissociated suspensions, to produce a monolayer, preparation is simplified and a cell culture that retains in vivo reactivity is formed.

Particularly, growth of malignant cells is optimised, without overgrowth of fibroblasts or other cells as often occurs in suspension cultures. The monolayers can be grown in a few weeks, contrast longer times required with single cell progeny by dilution cloning.

CHOSEN-DRAWING: Dwg.0/0 Dwg.0/0

TITLE-TERMS: ASSESS CHEMICO SENSITIVE PATIENT CELL MONO LAYER GROW MULTICELL PARTICLE SAMPLE RELATED VIVO BEHAVE SELECT AGENT TREAT TUMOUR INDIVIDUAL PATIENT

DERWENT-CLASS: B04 D16

CPI-CODES: B04-F02; B11-C08E; B12-K04A; D05-H09;

CHEMICAL-CODES:

Chemical Indexing M1 *01*
  Fragmentation Code
  M423 M750 M903 N102 N136 P633 P942 P943 Q233 V754

Chemical Indexing M6 *02*
  Fragmentation Code
  M903 P633 P831 P942 P943 Q233 R515 R521 R627 R639

SECONDARY-ACC-NO:
CPI Secondary Accession Numbers: C1998-036204

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L3: Entry 3 of 5   File: USPT   Mar 17, 1998

US-PAT-NO: 5728541
DOCUMENT-IDENTIFIER: US 5728541 A

TITLE: Method for preparing cell cultures from biologial specimens for chemotherapeutic and other assays

DATE-ISSUED: March 17, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kornblith; Paul L.	Pittsburgh	PA		

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Precision Therapeutics, Inc.	Pittsburgh	PA			02

APPL-NO: 08/ 679056   [PALM]
DATE FILED: July 12, 1996

INT-CL: [06] ·C12 Q 1/02, C12 Q 1/18

US-CL-ISSUED: 435/29; 435/30, 435/32, 435/261
US-CL-CURRENT: 435/29; 435/261, 435/30, 435/32

FIELD-OF-SEARCH: 435/2, 435/29, 435/30, 435/32, 435/240.2, 435/261, 435/803

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

[Search Selected] [Search ALL]

	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
☐	4423145	December 1983	Stampfer et al.	435/32
☐	4937187	June 1990	Rotman	435/30
☐	5242806	September 1993	Yen-Maguire et al.	435/32
☐	5270172	December 1993	Morgan	435/29
☐	5443950	August 1995	Naughton et al.	435/1

OTHER PUBLICATIONS

Cancer Research vol. 55; Julia T. Arnold. Betty P. Wilkinson. Sheela Sharma, and Vernon E. Steele; `Evaluation of Chemopreventive Agents in Different Mechanistic Classes Using a Rat Tracheal Epithelial Cell Culture Transformation Assay.sup.1 `; Feb. 1, 1995; pp. 537-543.
Anticancer Research vol. 13; Anna Kruczynski and Robert Kiss; `Evidence of a Direct Relationship Between the Increase in the in Vitro Passage Number of Human Non-Small-Cell-Lung cancer Primocultures and their Chemosensitivity`; 1993; pp. 507-514.

ART-UNIT: 121

PRIMARY-EXAMINER: Gitomer; Ralph

ABSTRACT:

An improved system for screening a multiple of candidate therapeutic or
chemotherapeutic agents for efficacy as to a specific patient, in which a tissue
sample from the patient is harvested, cultured and separately exposed to a plurality
of treatments and/or therapeutic agents for the purpose of objectively identifying
the best treatment or agent for the particular patient. The system includes the
initial preparation of cohesive multicellular particulates of the tissue sample,
rather than enzymatically dissociated cell suspensions or preparations, for initial
tissue culture monolayer preparation. Practical monolayers of cells may thus be
formed to enable meaningful screening of a plurality of treatments and/or agents. By
subjecting uniform samples of cells to a wide variety of active agents (and
concentrations thereof), the most promising agent and concentration for treatment of
a particular patient can be determined. For assays concerning cancer treatment, a
two-stage evaluation is contemplated in which both acute cytotoxic and longer term
inhibitory effect of a given anti-cancer agent are investigated.

7 Claims, 0 Drawing figures
Exemplary Claim Number: 1

BRIEF SUMMARY:

1   FIELD OF THE INVENTION

2   The invention relates to screening and testing of active agents, including
     chemotherapeutic agents, to predict potential efficacy in individual patients
     in whom treatment with such agents is indicated.

3   INTRODUCTION

4   All active agents including chemotherapeutic active agents are subjected to
     rigorous testing as to efficacy and safety prior to approval for medical use in
     the United States. Methods of assessing efficacy have included elaborate
     investigations of large populations in double blind studies as to a given
     treatment method and/or active agent, with concomitant statistical
     interpretation of the resulting data, but these conclusions are inevitably
     generalized as to patient populations taken as a whole. In many pharmaceutical
     disciplines and particularly in the area of chemotherapy, however, the results
     of individual patient therapy may not comport with generalized data--to the
     detriment of the individual patient. The need has been long recognized for a
     method of assessing the therapeutic potential of active agents, including but
     not limited to chemotherapeutic agents, for their efficacy as to a given
     individual patient, prior to the treatment of that patient.

5   Prior art assays already exist which expose malignant tissue of various types
     to a plurality of active agents, for the purpose of assessing the best choice
     for therapeutic administration. For example, in Kruczynski, A., et al.,
     "Evidence of a direct relationship between the increase in the in vitro passage
     number of human non-small-cell-lung cancer primocultures and their
     chemosensitivity," Anticancer Research, vol. 13, no. 2, pp. 507-513 (1993),
     chemosensitivity of non-small-cell-lung cancers was investigated in in vivo
     grafts, in in vitro primocultures and in commercially available long-term
     cancer cell lines. The increase in chemosensitivity was documented and
     correlated with morphological changes in the cells in question. Sometimes
     animal model malignant cells and/or established cell cultures are tested with
     prospective therapy agents, see for example Arnold, J. T., "Evaluation of
     chemopreventive agents in different mechanistic classes using a rat tracheal
     epithelial cell culture transformation assay," Cancer Res., vol. 55, no. 3, pp.
     537-543 (1995).

6   When actual patient cells are used to form in vitro assays focussed on
individual patients, in typical prior art processes the cells are harvested
(biopsied) and trypsinized (connective tissue digested with the enzyme trypsin)
to yield a cell suspension suitable for conversion to the desired·tissue
culture form. The in vitro tissue culture cell collections which result from
these techniques are generally plagued by their inability accurately to imitate
the chemosensitivity of the original tumor or other cell biopsy. Standard
cloning and tissue culture techniques are moreover excessively complicated and
expensive for use in a patient-by-patient assay setting. A need thus remains
for a technique of tissue culture preparation which provides cell cultures, for
drug screening purposes, in which after simple preparation the cell cultures
react in a manner equivalent to their in vivo reactivity, to enable drug or
chemotherapeutic agent screening as to a particular patient for whom such
screening is indicated.

7   SUMMARY OF THE INVENTION

8   In order to meet this need, the present invention is an improved system for
screening a multiple of candidate therapeutic or chemotherapeutic agents for
efficacy as to a specific patient, in which a tissue sample from the patient is
harvested, cultured and separately exposed to a plurality of treatments and/or
therapeutic agents for the purpose of objectively identifying the best
treatment for the cultured cells obtained from the patient. Specific method
innovations such as tissue sample preparation techniques render this method
practically as well as theoretically useful. One particularly important tissue
sample preparation technique is the initial preparation of cohesive
multicellular particulates of the tissue sample, rather than enzymatically
dissociated cell suspensions or preparations, for initial tissue culture
monolayer preparation. With respect to the culturing of malignant cells, for
example, it is believed (without any intention of being bound by the theory)
that by maintaining the malignant cells within a multicellular particulate of
the originating tissue, growth of the malignant cells themselves is facilitated
versus the overgrowth of fibroblasts or other cells which tends to occur when
suspended tumor cells are grown in culture. Practical monolayers of cells may
thus be formed to enable meaningful screening of a plurality of treatments
and/or agents. Growth of cells is monitored to ascertain the time to initiate
the assay and to determine the growth rate of the cultured cells; sequence and
timing of drug addition is also monitored and optimized. By subjecting uniform
samples of cells to a wide variety of active agents (and concentrations
thereof), the most efficacious agent can be determined. For assays concerning
cancer treatment, a two-stage evaluation is contemplated in which both acute
cytotoxic and longer term inhibitory effect of a given anti-cancer agent are
investigated.

DETAILED DESCRIPTION:

1   DETAILED DESCRIPTION OF THE INVENTION

2   The present invention is a system for screening a multiple of candidate
therapeutic or chemotherapeutic agents for efficacy as to a specific patient,
in which a tissue sample from the patient is harvested and separately exposed
to a plurality of treatments and/or therapeutic agents for the purpose of
objectively identifying the best treatment or agent. Specific method
innovations such as tissue sample preparation techniques render this method
practically as well as theoretically useful. One particularly important tissue
sample preparation technique is the initial preparation of cohesive
multicellular particulates (explants) of the tissue sample, rather than
enzymatically dissociated cell suspensions or preparations, for initial tissue
culture monolayer preparation. Cell growth, and sequence and timing of drug
addition, are monitored and optimized.

3   An important application of the present invention is the screening of chemotherapeutic agents and other antineoplastic therapies against tissue culture preparations of malignant cells from the patients from whom malignant samples are biopsied. Related anti-cancer therapies which can be screened using the inventive system are both radiation therapy and agents which enhance the cytotoxicity of radiation, as well as immunotherapeutic anti-cancer agents. Screening processes for treatments or therapeutic agents for nonmalignant syndromes are also embraced within this invention, however, and include without limitation agents which combat hyperproliferative syndromes, such as psoriasis, or wound healing agents. Nor is the present efficacy assay limited only to the screening of active agents which speed up (healing) or slow down (anti-cancer, anti-hyperproliferative) cell growth because agents intended to enhance or to subdue intracellular biochemical functions may be tested in the present tissue culture system also. For example, the formation or blocking of enzymes, neurotransmitters and other biochemicals may be screened with the present assay methods prior to treatment of the patient.

4   When the patient is to be treated for the presence of tumor, in the preferred embodiment of the present invention a tumor biopsy of >100 mg of non-necrotic, non-contaminated tissue is harvested from the patient by any suitable biopsy or surgical procedure known in the art. Biopsy sample preparation generally proceeds as follows under a Laminar Flow Hood which should be turned on at least 20 minutes before use. Reagent grade ethanol is used to wipe down the surface of the hood prior to beginning the sample preparation. The tumor is then removed, under sterile conditions, from the shipping container and is minced with sterile scissors. If the specimen arrives already minced, the individual tumor pieces should be divided into four groups. Using sterile forceps, each undivided tissue quarter is then placed in 3 ml sterile growth medium (Standard F-10 medium containing 17% calf serum and a standard amount of Penicillin and Streptomycin) and systematically minced by using two sterile scalpels in a scissor-like motion, or mechanically equivalent manual or automated opposing incisor blades. This cross-cutting motion is important because the technique creates smooth cut edges on the resulting tumor multicellular particulates. Preferably but not necessarily, the tumor particulates each measure 1 mm.sup.3. After each tumor quarter has been minced, the particles are plated in culture flasks using sterile pasteur pipettes (9 explants per T-25 or 20 particulates per T-75 flask). Each flask is then labelled with the patient's code, the date of explantation and any other distinguishing data. The explants should be evenly distributed across the bottom surface of the flask, with initial inverted incubation in a 37.degree. C. incubator for 5-10 minutes, followed by addition of about 5-10 ml sterile growth medium and further incubation in the normal, non-inverted position. Flasks are placed in a 35.degree. C., non-CO.sub.2 incubator. Flasks should be checked daily for growth and contamination. Over a period of a few weeks, with weekly removal and replacement of 5 ml of growth medium, the explants will foster growth of cells into a monolayer. With respect to the culturing of malignant cells, it is believed (without any intention of being bound by the theory) that by maintaining the malignant cells within a multicellular particulate of the originating tissue, growth of the malignant cells themselves is facilitated versus the overgrowth of fibroblasts (or other unwanted cells) which tends to occur when suspended tumor cells are grown in culture.

5   The use of the above procedure to form a cell monolayer culture maximizes the growth of malignant cells from the tissue sample, and thus optimizes ensuing tissue culture assay of chemotherapeutic action of various agents to be tested. Enhanced growth of actual malignant cells is only one aspect of the present invention, however; another important feature is the growth rate monitoring system used to oversee growth of the monolayer once formed. Once a primary culture and its derived secondary monolayer tissue culture has been initiated, the growth of the cells is monitored to ascertain the time to initiate the chemotherapy assay and to determine the growth rate of the cultured cells.

6   Monitoring of the growth of cells is conducted by counting the cells in the monolayer on a periodic basis, without killing or staining the cells and

without removing any cells from the culture flask. The counting may be done visually or by automated methods, either with or without the use of estimating techniques known in the art (counting in a representative area of a grid multiplied by number of grid areas, for example). Data from periodic counting is then used to determine growth rates which may or may not be considered parallel to growth rates of the same cells in vivo in the patient. If growth rate cycles can be documented, for example, then dosing of certain active agents can be customized for the patient. The same growth rate can be used to evaluate radiation treatment periodicity, as well. It should be noted that with the growth rate determinations conducted while the monolayers grow in their flasks, the present method requires no hemocytometry, flow cytometry or use of microscope slides and staining, with all their concomitant labor and cost.

7 Protocols for monolayer growth rate generally use a phase-contrast inverted microscope to examine culture flasks incubated in a 37.degree. C. (5% CO.sub.2) incubator. When the flask is placed under the phase-contrast inverted microscope, ten fields (areas on a grid inherent to the flask) are examined using the 10.times. objective, with the proviso that the ten fields should be non-contiguous, or significantly removed from one another, so that the ten fields are a representative sampling of the whole flask. Percentage cell occupancy for each field examined is noted, and averaging of these percentages then provides an estimate of overall percent confluency in the cell culture. When patient samples have been divided between two or among three or more flasks, an average cell count for the total patient sample should be calculated. The calculated average percent confluency should be entered into a process log to enable compilation of data--and plotting of growth curves--over time. Monolayer cultures may be photographed to document cell morphology and culture growth patterns. The applicable formula is: ##EQU1## As an example, therefore, if the estimate of area occupied by the cells is 30% and the total area of the field is 100%, percent confluency is 30/100, or 30.

8 Adaptation of the above protocol for non-tumor cells is straightforward and generally constitutes an equivalent procedure.

9 Active agent screening using the cultured cells does not proceed in the initial incubation flask, but generally proceeds using plates such as microtiter plates. The performance of the chemosensitivity assay used for screening purposes depends on the ability to deliver a reproducible cell number to each row in a plate and/or a series of plates, as well as the ability to achieve an even distribution of cells throughout a given well. The following procedure assures that cells are reproducibly transferred from flask to microtiter plates, and cells are evenly distributed across the surface of each well.

10 The first step in preparing the microtiter plates is, of course, preparing and monitoring the monolayer as described above. The following protocol is exemplary and susceptible of variation as will be apparent to one skilled in the art. Cells are removed from the culture flask and a cell pellet is prepared by centrifugation. The cell pellet derived from the monolayer is then suspended in 5 ml of the growth medium and mixed in a conical tube with a vortex for 6 to 10 seconds. The tube is then rocked back and forth 10 times. A 36 .mu.l droplet from the center of the conical tube is pipetted onto one well of a 96 well plate. A fresh pipette is then used to pipette a 36 .mu.l aliquot of trypan blue solution, which is added to the same well, and the two droplets are mixed with repeated pipette aspiration. The resulting admixture is then divided between two hemocytometer chambers for examination using a standard light microscope. Cells are counted in two out of four hemocytometer quadrants, under 10.times. magnification. Only those cells which have not taken up the trypan blue dye are counted. This process is repeated for the second counting chamber. An average cell count per chamber is thus determined. Using means known in the art, the quadrant count values are checked, logged, multiplied by 10.sup.4 to give cells/ml, and the total amount of fluid (growth medium) necessary to suspend remaining cell aliquots is calculated accordingly.

11 After the desired concentration of cells in medium has been determined,

additional cell aliquots from the monolayer are suspended in growth medium via vortex and rocking and loaded into a Terasaki dispenser known in the art. Aliquots of the prepared cell suspension are delivered into the microtiter plates using Terasaki dispenser techniques known in the art. A plurality of plates may be prepared from a single cell suspension as needed. Plates are then wrapped in sterile wet cotton gauze and incubated in an incubator box by means known in the art.

12   After the microtiter plates have been prepared, exposure of the cells therein to active agent is conducted according to the following exemplary protocol. During this portion of the inventive assay, the appropriate amount of specific active agent is tranferred into the microtiter plates prepared as described above. A general protocol, which may be adapted, follows. Each microtiter plate is unwrapped from its wet cotton gauze sponge and microscopically examined for cell adhesion. Control solution is dispensed into delineated rows of wells within the grid in the microtiter plate, and appropriate aliquots of active agent to be tested are added to the remaining wells in the remaining rows. Ordinarily, sequentially increasing concentrations of the active agent being tested are administered into progressively higher numbered rows in the plate. The plates are then rewrapped in their gauze and incubated in an incubator box at 37.degree. C. under 5% CO.sub.2. After a predefined exposure time, the plates are unwrapped, blotted with sterile gauze to remove the agent, washed with Hank's Balance Salt Solution, flooded with growth medium, and replaced in the incubator in an incubator box for a predefined time period, after which the plates may be fixed and stained for evaluation.

13   Fixing and staining may be conducted according to a number of suitable procedures; the following is representative. After removal of the plates from the incubator box, culture medium is poured off and the plates are flooded with Hank's Balance Salt Solution. After repeated flooding (with agitation each time) the plates are then flooded with reagent grade ethanol for 2-5 minutes. The ethanol is then poured off. Staining is accomplished with approximately 5 ml of Giemsa Stain per plate, although volume is not critical and flooding is the goal. Giemsa stain should be left in place 5 min. .+-.30 seconds as timing influences staining intensity. The Giemsa stain is then poured off and the plates are dipped 3 times cold tap water in a beaker. The plates are then inverted, shaken vigorously, and air dried overnight (with plate lids off) on a rack on a laboratory bench. Cells per well are then counted manually or by automated and/or computerized means, to derive data regarding chemosensitivity of cells at various concentrations of exposure. One particularly useful computer operating environment for counting cells is the commercially available OPTIMATE compiler, which is designed to permit an optical counting function well suited to computerized cell counting procedures and subsequent calculations.

14   The above procedures do not change appreciably when cell growth promoters are assayed rather than cell arresting agents such as chemotherapeutic agents. The present assay allows cell death or cell growth to be monitored with equal ease. In any case, optimization of use of the present system will involve the comparative testing of a variety of candidate active agents, for selection of the best candidate for patient treatment based upon the in vitro results. One particularly advantageous embodiment of the above described invention comprises a two-stage assay for cytotoxicity followed by evaluation of longer-term inhibitory effect. Chemotherapeutic agents may thus be evaluated separately for both their direct chemotherapeutic effect as well as for their longer duration efficacy.

15   Identification of one or more active agents or chemotherapeutic agents is peripheral to the present invention, which is intended for the efficacy screening of any or all of them as to a given patient. Literally any active agent may be screened according to the present invention; listing exemplary active agents is thus omitted here.

16   The essence of the invention thus includes the important feature of the

simplicity of the present system--cohesive multicellular particulates of the patient tissue to be tested are used to form cell monolayers; growth of those monolayers is monitored for accurate prediction of correlating growth of the same cells in vivo; and differing concentrations of a number of active agents may be tested for the purpose of determining not only the most appropriate agent but the most appropriate concentration of that agent for actual patient exposure (according to the calculated cell growth rates). It is also important to note, in the context of the invention, that the present system allows in vitro tests to be conducted in suspensions of tissue culture monolayers grown in nutrient medium under fast conditions (a matter of weeks), rather than with single cell progeny produced by dilution cloning over long periods of time. In some cases, the present invention is a two stage assay for both cytotoxicity and the longer-term growth inhibitory.

17   Although the present invention has been described with respect to specific materials and methods above, the invention is only to be considered limited insofar as is set forth in the accompanying claims.

CLAIMS:

I claim:

1. A method for preparing a biopsy sample of tissue containing malignant cells for chemosensitivity testing, comprising:

(a) obtaining a tumor specimen;

(b) mechanically separating said specimen into cohesive multicellular particulates having a size of about 1 mm.sup.3 ;

(c) growing a tissue culture monolayer from said cohesive multicellular particulates;

(d) inoculating cells from said monolayer into a plurality of segregated sites;

(e) treating said plurality of sites with at least one agent;

(f) examining said plurality of sites; and

(g) assessing chemosensitivity of the cells in said plurality sites.

2. The method according to claim 1 wherein said plurality of segregated sites comprise a plate containing a plurality of wells therein.

3. The method according to claim 2 wherein said plurality of wells is treated over a length of time adequate to permit assessment of both initial cytotoxic effect and longer-term inhibitory effect of said at least one agent.

4. The method according to claim 3 wherein step (d) is accomplished by a multiple well pipetting device.

5. The method according to claim 4 wherein the cells in step (d) are further suspended in medium prior to inoculation into said plate containing a plurality of wells therein.

6. The method according to claim 5 wherein said agent is at least one of the agents selected from group consisting of a radiation therapy agent, a radiation therapy sensitizing agent and a radiation therapy desensitizing agent.

7. The method according to claim 5 wherein said agent is an immunotherapeutic agent.

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L2: Entry 1 of 2   File: USPT   Jul 9, 2002

US-PAT-NO: 6416967
DOCUMENT-IDENTIFIER: US 6416967 B2

TITLE: Method of using multicellular particulates to analyze malignant or
hyperproliferative tissue

DATE-ISSUED: July 9, 2002

INVENTOR-INFORMATION:

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APPL-NO: 09/ 189310   [PALM]
DATE FILED: November 10, 1998

PARENT-CASE:
RELATED APPLICATION This is a Continuation-In-Part of U.S. application Ser. No.
08/679,056, filed Jul. 12, 1996, now U.S. Pat. No. 5,728,541, granted Mar. 17, 1998;
U.S. application Ser. No. 09/095,993, filed Jun. 11, 1998; and U.S. application Ser.
No. 09/039,957, filed Mar. 16, 1998.

INT-CL: [07] C12 Q 1/02

US-CL-ISSUED: 435/29; 435/30
US-CL-CURRENT: 435/29; 435/30

FIELD-OF-SEARCH: 435/29, 435/30, 435/32, 435/240.2, 435/261, 435/803

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

| Search Selected | | Search ALL |

PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
☐ 4423145	December 1983	Stampfer et al.	435/32
☐ 4937187	June 1990	Rotman	435/30
☐ 5242806	September 1993	Yen-Maguire et al.	435/32
☐ 5270172	December 1993	Morgan	435/29
☐ 5607918	March 1997	Eriksson et al.	514/12
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☐ 5874218	February 1999	Drolet et al.	435/6
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"Evaluation of Chemopreventive Agents in Different Mechanistic Classes Using a Rat Tracheal Epithelial Cell Cultrue Transformation Assay." Arnold et al., Cancer Research, vol. 55, pp. 537-543 (1995).
"Evidence of a Direct Relationship Between the Increase in the In Vitro Passage Number of Human Non-Small-Cell-Lung Cancer Primocultures and Their Chemosensitivity," Kruczynski et al., Anticancer Research, vol. 13, pp. 507-514 (1993).
Arnold, J. Evaluation of Chemopreventive Agents in Different Mechanistic Classes Using a Rat Tracheal Epithelial Cell Culture Transformation Assay. Cancer Research 55:537-543, Feb. 1995.*
Kruczynski A. Evidence of a Direct Relationship Between the Increase in the in Vitro Passage Number of Human Non-Small-Cell Lung Cancer Primocultures and Their Chemosensitivity. Anticancer Research 13:507-514, 1993.

ART-UNIT: 1623

PRIMARY-EXAMINER: Gitomer; Ralph

ABSTRACT:

A comprehensive and integrated system for monitoring (identifying, tracking and analyzing) an individual patient's malignancy or hyperproliferative syndrome through the duration of a malignancy or hyperproliferative syndrome as to a specific patient is provided. Specimens of a patient's cells are collected and mechanically separated into cohesive multicellular particulates. A tissue culture monolayer is grown from the multicellular particulates to form a prime culture, and the tissue culture is monitored over a period of time. The invention allows for study of the effect of various treatment methods on cellular production of vascular endothelial growth factor.

22 Claims, 34 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 8

BRIEF SUMMARY:

1   BACKGROUND OF THE INVENTION

2   Field of the Invention

3   A system is provided for in vitro tracking of cancerous tissue over the course

of the malignancy. The system provides a method for identifying the malignancy and for determining a patient's prognosis. Further, the system provides for assessing a malignancy's invasiveness, aggressiveness, growth rate, production of extracellular markers, possible side effects and for determining the efficacy on the malignancy of a given therapeutic regimen. The system also allows for generation of a therapeutic index, which serves as an indicator of a given therapy's effectiveness against the malignancy as compared to its undesirable side effects, such as lethality to a patient's normal cells.

4   INTRODUCTION

5   Tracking a malignancy in a patient according to prior art methods is an inaccurate process which involves identification of the malignancy through techniques including biopsy and subsequent histological, biochemical, and immunochemical techniques and regularly monitoring the malignancy's progression by invasive (i.e., biopsy) or noninvasive (i.e., x-ray, nuclear imaging, Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET)) methods. These methods are often expensive, inconvenient, painful and usually involve hospital visits and safety risks. It is, therefore, desirable to reduce a patient's exposure to such methods. Furthermore, identification of a malignancy as a known variety of malignancy is often helpful in determining a suitable therapeutic approach and expected prognosis. However, even individually identifiable malignancies differ from patient-to-patient in their growth characteristics and in their responsiveness to treatment.

6   Determination of the growth rate, invasiveness and aggressiveness of a given malignancy is critical to prognosis and to the choice of therapies. A patient with a poor prognosis might be given a therapeutic regimen which might be more effective than another regimen but more risky to the patient. A patient with a better prognosis might be given a therapeutic regimen which is less aggressive and less risky to the patient, but which might not be as effective as often as a more dangerous therapy. Similarly, if a malignancy produces factors or creates conditions which cause a dangerous side effect, such as a thrombogenesis, the patient can be treated, preferably prophylactically, for the condition.

7   Current methodologies for determining growth rate, invasiveness, aggressiveness or which track the progression of a malignancy include biopsy and short-term culture, which can include drawing of blood or other bodily fluids, or semi- or non-invasive techniques such as x-ray and nuclear imaging. At any given time, a patient could be subject to multiple procedures, depending upon when the information is needed by the physician. Each procedure requires the presence of the patient and usually creates risk or pain. These procedures also can increase the stress level of the patient, which often is an exacerbating factor in cancer and associated prognoses. It is therefore, desirable to reduce the frequency of such procedures.

8   Identification of an effective therapeutic regimen is critically important to a patient. Often, once the malignancy is identified, a therapy is chosen based upon prior research on that type of malignancy and is not tailored to the sensitivities of the malignancy of a given patient. Often secondary therapies are needed because a first choice was ineffective. Valuable treatment time can be lost and a patient's life can be threatened.

9   All active agents including chemotherapeutic active agents are subjected to rigorous testing as to efficacy and safety prior to approval for medical use in the United States. Methods of assessing efficacy have included elaborate investigations of large populations in double blind studies as to a given treatment method and/or active agent, with concomitant statistical interpretation of the resulting data, but these conclusions are inevitably generalized as to patient populations taken as a whole. In many pharmaceutical disciplines and particularly in the area of chemotherapy, however, the results of individual patient therapy may not comport with generalized data--to the detriment of the individual patient. The need has been long recognized for a

method of assessing the therapeutic potential of active agents, including but not limited to chemotherapeutic agents, for their efficacy as to a given individual patient, prior to the treatment of that patient. This need also applies to assessing the therapeutic potential as to radiation therapies, combined radiation/drug therapies and cellular immunotherapies.

10   Prior art assays already exist which expose malignant tissue of various types to a plurality of active agents, for the purpose of assessing the best choice for therapeutic administration. For example, in Kruczynski, A., et al., "Evidence of a direct relationship between the increase in the in vitro passage number of human non-small-cell-lung cancer primocultures and their chemosensitivity," Anticancer Research, vol. 13, no. 2, pp. 507-513 (1993), chemosensitivity of non-small-cell-lung cancers was investigated in vivo grafts, in vitro primocultures and in commercially available long-term cancer cell lines. The increase in chemosensitivity was documented and correlated with morphological changes in the cells in question. Sometimes animal model malignant cells and/or established cell cultures are tested with prospective therapy agents, see for example Arnold, J. T., "Evaluation of chemopreventive agents in different mechanistic classes using a rat tracheal epithelial cell culture transformation assay," Cancer Res., vol. 55, no. 3, pp. 537-543 (1995).

11   In vitro prior art techniques present the further shortcoming that assayed cells do not necessarily express the cellular markers they would express in vivo. This is regrettable because the determination of expression of certain secreted or cellular markers, secreted factors or tumor antigens or lack thereof can be useful for both identification and therapeutic purposes. For instance, members of the fibrinolytic system such as urokinase-type plasminogen activator (u-PA) and plasminogen activator inhibitors type 1 (PAI-1) are up-regulated in malignant brain tumors. See, e.g., Jasti S. Rao, et al., "The Fibrinolytic System in Human Brain Tumors: Association with Pathophysiological Conditions of Malignant Brain Tumors," Advances in Neuro-Oncology II, Kornblith P L, Walker M D (eds) Futura (1997). Other secreted factors such as .alpha.-fetoprotein, carcinoembryonic antigen and transforming growth factors .alpha. and .beta. have been found to be indicative of various cancers and/or cancer progression (see also, Singhal et al., "Elevated Plasma Osteopontin in Metastatic Breast Cancer Associated with Increased Tumor Burden and Decreased Survival," Clinical Cancer Research, vol. 3, 605-611, (April 1997); Kohno et al., "Comparative Studies of CAM 123-6 and Carcinoembryonic Antigen for the Serological Detection of Pulmonary Adenocarcinoma," Cancer Detection and Prevention, 21 (2): 124-128 (1997)). These examples are but a few of the many factors that may be used to identify diseased cells.

12   Cellular markers also include metastatic markers, indicative of metastatic potential, i.e., invasiveness and aggressiveness, which is relevant to the progression of a given malignancy and to a patient's prognosis. First, markers indicating the invasiveness of a given malignancy indicate the ability of the malignancy to infiltrate and to destroy adjacent tissue. As an example, for epithelial malignancies, invasiveness markers are indicative of the ability of the malignancy to infiltrate beneath the epithelial basement membrane. Invasiveness markers can include the presence of proteolytic enzymes or angiogenic factors. A second category of metastatic marker indicates growth conditions of the malignancy. For instance, a malignancy could require for instance a prostate-specific factor for growth. Invasiveness and aggressiveness factors are often present in serum or in tissue culture media.

13   Relevant to a patient's prognosis and, incidentally, to the identification of a malignancy is the presence of markers, cellular or secreted, which lead to complications beyond those involved with uncontrolled growth and invasion by a malignancy. For instance, secretion by the malignancy of thrombogenic substances by the malignancy can result in blood clotting, resulting in thrombophlebitis or other thrombotic events such as pulmonary thrombosis. Identification of a thrombotic potential indicates treatment (preferably prophylactically) with thrombolytic substances.

14   When a specific patient's cells are used in vitro assays in typical prior art processes the cells are harvested (biopsied) and trypsinized (connective tissue digested with the enzyme trypsin) to yield a cell suspension purportedly suitable for conversion to the desired tissue culture form. The in vitro tissue culture cell collections which result from these techniques are generally plagued by their inability accurately to imitate the chemosensitivity or therapeutic sensitivity of the original tumor or other cell biopsy. These collections often do not express cellular markers in the same manner that they would in vivo. A need thus remains for a technique of tissue culture preparation which provides cell cultures, allowing identification of a malignancy, accurate tracking of the malignancy's progress in a patient and therapy screening, in which, after simple preparation, the cell cultures react in a manner equivalent to their in vivo reactivity. The culture method would enable drug or chemotherapeutic agent, radiation therapy and/or cellular immunotherapy screening as to a particular patient for whom such screening is indicated.

15   A need also remains for a technique of tissue culture preparation which provides cell cultures for screening for expressed markers or factors where the cultured cells express the markers or factors in a manner indicative of their in vivo expression of the same. A further need also remains for a tissue culture preparation which allows for morphological study of the cells. Lastly, a need remains for a tissue culture system in which progression of an individual malignancy can be studied as indicative of the in vivo progression of the malignancy.

16   SUMMARY OF THE INVENTION

17   A comprehensive and integrated unified system for monitoring (i.e., identifying, tracking and analyzing) an individual patient's malignancy through the duration of a malignancy as to a specific patient is provided. The method of the present invention allows for initial identification of a malignancy, identification of malignancy-specific cellular or secreted markers, identification of cellular or secreted markers indicative of complications, study of the invasiveness and aggressiveness of the malignancy, study of the growth rate of the malignancy, study of the effect of therapies on the malignancy as compared to control cells of the same patient (chemosensitivity versus toxicity) and the identification of a therapeutic index (i.e., the ratio of chemosensitivity:toxicity), study of tumor morphology and study of histological and cytochemical markers.

18   The method of the present invention includes the steps of collecting a tissue sample or specimen of a patient's cells and separating the specimen into cohesive multicellular particulates (explants) of the tissue sample, rather than enzymatically digested cell suspensions or preparations. The cells are then grown as a tissue culture monolayer from the multicellular particulates to form a prime culture. A specimen can be taken from a patient at any relevant site, including but not limited to tissue, ascites or effusion fluid. Samples may also be taken from body fluid or exudates, as is appropriate. A tissue culture monolayer, designated as the prime culture, can be grown in any method known in the art for growing such a monolayer, for instance in tissue culture plates or flasks. If the malignant cells originate from solid tissue, however, the tissue must be subdivided into small pieces from which a tissue culture monolayer is then grown out.

19   Once a prime culture is established from a patient's malignancy, the prime culture can be maintained without any treatments beside normal feedings and passage techniques, as indicative of the growth of the malignancy absent treatment. However, subcultures of the prime culture are prepared so that the prime culture is preferably left untreated, and the cells of the prime culture are not affected by any testing. However, either the prime culture or a subculture thereof can be propagated as a reference culture. The reference culture is a culture which is treated with therapies reflective of a patient's actual treatments. For instance, if a patient is treated with a

chemotherapeutic agent, the reference culture is treated with the same agent in the same concentration. The reference culture can be monitored genotypically or phenotypically to reflect actual progress of the malignancy in the patient. Treatment of the reference culture need not be limited to anticancer therapies, but can reflect all of a patient's treatments. For instance, thrombolytic or anti-thrombogenic treatments can be applied to the reference culture to reflect a patient's treatment. Subcultures of either the prime culture or the reference culture can be used for further analysis. Preferably, since the reference culture is indicative of the current state of a malignancy at a given time, subcultures of the reference culture are analyzed further. At various points in the passage of the control culture and the reference culture, aliquots of cells from those cultures can be stored cryogenically or otherwise.

20   The tissue sample technique of the present invention is also useful in assaying expression and/or secretion of various markers, factors or antigens present on or produced by the cultured cells. These assays can be used for diagnostic purposes for monitoring the applicability of certain candidate therapeutic or chemotherapeutic agents or for monitoring the progress of treatment of the cancer with those agents.

21   A method for identifying and monitoring progress of a malignancy in an individual patient is provided including the steps of inoculating cells from either the prime culture, the reference culture or a subculture of the prime culture or of the reference culture into a plurality of segregated sites; treating the plurality of sites with at least one treating means or therapy, followed by assessment of sensitivity of cells in the site to the treating means; collecting a specimen of a patient's non-malignant cells; separating the non-malignant cells into cohesive multicellular particles; growing a tissue culture monolayer from the multicellular particulates of non-malignant cells to form a control culture; inoculating the control culture in a plurality of non-segregated sites; treating the plurality of segregated sites of the control culture with the same treating means as the segregated sites of the prime culture or a subculture thereof, followed by assessment of the sensitivity of the segregated cells of the control culture to the treating means; and comparing the sensitivity of the segregated cells of the prime culture or a subculture thereof with the sensitivity of the segregated cells of the control culture to the treating means. The assessments described above are calculations of the percentage or fraction of cells sensitive, or insensitive, to the treatment and the method further includes the step of creating a therapeutic index of a ratio of one of the percentage of or the fraction of sensitive cells or insensitive cells in the segregated cells of the control culture to one of the percentage of or the fraction of sensitive cells or insensitive cells in the segregated cells of the prime culture or subculture thereof.

22   Lastly, a method for treating a patient having a malignancy is provided, including the steps of: analyzing a patient's cells prepared according to the above-described methods for malignancy-associated markers; determining a therapeutic regimen according to the results of the analysis; and treating a patient according to the regimen. The method can further include the step of treating one of either cells cultured as a subculture of the prime culture or cells of the prime culture according to the regimen as representative of the patient's malignancy. Lastly, the method further includes determining a therapeutic index for each treating means as described above.

23   When applicable, cultures can be grown in a readable (scannable) plate and to determine percent confluence of the cells or any other parameter which can be determined in such a manner. The scanner can be operably linked with a computer or CPU to automatically input data into the computer or CPU. The computer or CPU can be programmed to automatically calculate a therapeutic index (or other relevant indices) based upon the data provided by the scanner. Alternatively, the data can be entered manually into the programmed computer or CPU to calculate the index.

DRAWING DESCRIPTION:

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A, 1B and 1C are graphs of the growth rates of three independent cell cultures.

FIGS. 2A-2F through 5A-5F are graphs depicting the results of short-term and long-term chemotherapy assays.

FIGS. 2A-2F and 3A-3F show short-term and long-term assays for a first patient.

FIGS. 4A-4F and 5A-5F show short-term and long-term assays for a second patient.

FIGS. 6 and 7 show two radiation dose versus surviving fraction curves for two glioblastoma cell lines. Cells were irradiated in microtiter plates and assayed four days post-irradiation.

FIGS. 8A-8C are graphs of survival rates of cell cultures treated with radiation (FIG. 8A) or with radiation and Taxol (FIGS. 8B and 8C).

FIGS. 9A and 9B are graphs showing data from a series of experiments where target cells from two tumor types were exposed to Activated Natural Killer (ANK) cells.

DETAILED DESCRIPTION:

1   DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

2   The present invention is an improved and unified system for monitoring the progression of an individual malignancy and for identifying cellular and secreted markers, markers indicative of certain side effects of the malignancy and for screening multiple candidate therapeutic or chemotherapeutic agents for efficacy and long term effect as to a specific patient. In the method, a tissue sample from the patient is harvested, cultured and separately exposed to a plurality of treatments and/or therapeutic agents for the purpose of objectively identifying the best treatment for the cultured cells obtained from the patient. The culture techniques of the present invention also result in a monolayer of cells that express cellular markers, secreted factors and tumor antigens in a manner representative of their expression in vivo. Specific method innovations such as tissue sample preparation techniques render this method practically as well as theoretically useful. One particularly important tissue sample preparation technique is the initial preparation of cohesive multicellular particulates of the tissue sample, rather than enzymatically dissociated cell suspensions or preparations, for initial tissue culture monolayer preparation. With respect to the culturing of malignant cells, for example, it is believed (without any intention of being bound by the theory) that by maintaining the malignant cells within a multicellular particulate of the originating tissue, growth of the malignant cells themselves is facilitated versus the overgrowth of fibroblasts or other cells which tends to occur when suspended tumor cells are grown in culture. Practical monolayers of cells may thus be formed to enable meaningful screening of a plurality of treatments and/or agents as well as meaningful identification of cellular markers. In the drug assays, growth of cells is monitored to ascertain the time to initiate the assay and to determine the growth rate of the cultured cells; sequence and timing of drug addition is also monitored and optimized. By subjecting uniform samples of cells to a wide variety of active agents (and concentrations thereof), the most efficacious agent can be determined. For assays concerning cancer treatment, a two-stage evaluation is contemplated in which both acute cytotoxic and longer term inhibitory effects of a given anti-cancer agent are investigated.

3   With regard to the identification of expressed cellular markers, secreted factors or tumor antigens, with the initial culturing of the multicellular

particulates it is believed (without any intention of being bound by the theory) that because the cells are grown under conditions closer to those found in vivo, the cells express their cellular markers, secreted factors and tumor antigens in a manner more closely resembling their expression in vivo. By assaying the culture media obtained from growing a monolayer according to the inventive method or by histochemically and/or immunohistochemically assaying the cells grown under such conditions, a more accurate profile of the cellular markers or factors is obtained.

4   Thus, a comprehensive and integrated system for identifying, tracking and analyzing an individual patient's malignancy through the duration of the malignancy and thereafter is provided. The method of the present invention allows for initial identification of a malignancy, identification of malignancy-specific cellular or secreted markers, identification of cellular or secreted markers indicative of complications, study of the invasiveness and aggressiveness of the malignancy, study of the growth rate of the malignancy, study of the effect of therapies on the malignancy as compared to control cells of the same patient (chemosensitivity and/or radiosensitivity versus toxicity) and the identification of a therapeutic index (i.e., the ratio of chemosensitivity:toxicity), study of tumor morphology and study of histological, cytochemical and immunocytochemical markers.

5   The method of the present invention includes the steps of collecting a tissue sample or specimen of a patient's cells and separating the specimen into cohesive multicellular particulates (explants) of the tissue sample, rather than enzymatically digested cell suspensions or preparations. The cells are then grown as a tissue culture monolayer from the multicellular particulates to form a prime culture. A specimen can be taken from a patient at any relevant site, including but not limited to tissue, ascites or effusion fluid. Samples may also be taken from body fluid or exudates, as is appropriate. A tissue culture monolayer can be grown in any method known in the art for growing such a monolayer, for instance in tissue culture plates or flasks.

6   Once a prime culture is established from a patient's malignancy, the prime culture can be maintained without any treatments beside normal feedings and passage techniques, as indicative of the growth of the malignancy absent treatment with a therapeutic regimen. Subcultures of the prime culture are prepared so that the cells of the prime culture are not affected by any subsequent testing or treatments. Although prime culture is preferably left untreated, either the prime culture or a subculture thereof can be propagated as a reference culture. The reference culture is a culture which is treated with therapies reflective of a patient's actual treatment regimen. For instance, if a patient is treated with a chemotherapeutic agent, the reference culture is treated with the same agent in the same concentration. The reference culture can be monitored genotypically or phenotypically to reflect actual progress of the malignancy in the patient. Treatment of the reference culture need not be limited to anticancer therapies, but can reflect all of a patient's treatments. For instance, thrombolytic or anti-thrombogenic treatments, can be applied to the reference culture to reflect a patient's treatment. Subcultures of either the prime culture or the reference culture can be used for further analysis. Preferably, since the reference culture is indicative of the current state in a patient of a malignancy, subcultures of the reference culture are analyzed. At various points in the passage of the control culture and the reference culture, aliquots of cells from those cultures can be stored cryogenically, or otherwise.

7   An important further aspect of the present invention is to provide a system for screening specific tissue samples from individual patients for expressed cellular markers, secreted factors or antigens, including tumor antigens, characteristic of the tissue sample. A tissue sample from a patient is harvested and grown in a monolayer culture as described above. Culture medium in which the cultures or subcultures thereof are assayed for the presence or absence of certain factors, such as secreted tumor antigens such as PAI-1, u-PA, cancer associated serum antigen (CASA) or carcinoembryonic antigen (CEA).

These factors may be detected through use of standard assays such as radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA), although other assays known to those skilled in the art may be used to detect and/or to quantify the soluble factors. The cell cultures grown in this manner may also be assayed histochemically and or immunohistochemically for identification or quantification of cellular or membrane-bound markers. Examples of such markers include, without limitation, CEA, tissue polypeptide specific antigen, (TPS) and mucin antigens, such as CA 15-3, CA 549, CA 27.29 and MCA. By screening tissue samples in this manner, for production of such factors, markers or antigens, the cultured cells may be further identified, aiding the physician in treatment strategies and as a prognosis indicator. Furthermore, by combining the use of the culture technique with assaying for such markers, factors and antigens, a treatment strategy for a disease state may be optimized and treatment progression may be monitored.

8   One important aspect of analyzing tissue culture medium is that complications of a malignancy can be predicted. For instance, one common complication is thrombogenesis. A propensity towards blood clot formation can be detected in tissue culture medium by identifying thrombogenic or procoagulant factors such as, without limitation, the Lewis Y antigen (Ley), HLA-DR and other tumor procoagulants, such as cancer procoagulant (CP) and tissue factor (TF). By identifying production of thrombogenic factors, a physician can prescribe drug and/or exercise regimens, as appropriate, to prevent life and/or limb-threatening clotting.

9   Cells and/or tissue culture media from any of the prime culture, the reference culture or subcultures thereof can be analyzed for tumor aggressiveness and invasiveness markers. Presence of these markers or absence thereof is highly relevant to a patient's prognosis. Furthermore, the effect of a given therapy on any of these markers can be analyzed. For instance, a tumor may produce angiogenic factors, such as, without limitation, vascular.endothelial growth factor (VEGF), which would lead a doctor to give a patient a less favorable prognosis. Other markers can include, without limitation, factors which allow cancer cells to affix to organs other than those from which the cancer cells derive, for instance, beta 3 integrin, which participates in the ability of melanoma cells to adhere to blood vessel walls. However, the effectiveness of therapies can be assessed if the presence of the angiogenic marker is analyzed in segregated sites according to the method of the present invention. A physician can suppress a malignancy by preventing expression of factors or markers which cause the malignancy's aggressiveness or invasiveness.

10   An important application of the present invention is the screening of chemotherapeutic agents and other antineoplastic therapies in tissue culture preparations of malignant cells from the patients from whom malignant samples are biopsied. Related anti-cancer therapies which also can be screened using the inventive system include radiation therapy and agents which enhance the cytotoxicity of radiation, as well as immunotherapeutic anti-cancer agents. Screening processes for treatments or therapeutic agents for nonmalignant syndromes are also embraced within this invention and include without limitation agents which combat hyper-proliferative syndromes, such as psoriasis, or wound healing agents. Nor is the present efficacy assay limited only to the screening of active agents which speed up (healing) or slow down (anti-cancer, anti-hyper-proliferative) cell growth because agents intended to enhance or to subdue intracellular biochemical functions may be tested in the present tissue culture system also. For example, the formation or blocking of enzymes, neurotransmitters and other biochemicals may be screened with the present assay methods prior to treatment of the patient.

11   When a patient is to be treated for the presence of tumor, in the preferred embodiment of the present invention a tumor biopsy of >100 mg of non-necrotic, non-contaminated tissue is harvested from the patient by any suitable biopsy or surgical procedure known in the art. Biopsy sample preparation generally proceeds as follows under a Laminar Flow Hood which should be turned on at least 20 minutes before use. Reagent grade ethanol is used to wipe down the

surface of the hood prior to beginning the sample preparation. The tumor is then removed, under sterile conditions, from the shipping container and is minced with sterile scissors. If the specimen arrives already minced, the individual tumor pieces should be divided into four groups. Using sterile forceps, each undivided tissue quarter is then placed in 3 ml sterile growth medium (Standard F-10 medium containing 17% calf serum and a standard amount of Penicillin and Streptomycin) and systematically minced by using two sterile scalpels in a scissor-like motion, or mechanically equivalent manual or automated opposing incisor blades. This cross-cutting motion is important because the technique creates smooth cut edges on the resulting tumor multicellular particulates. Preferably but not necessarily, the tumor particulates each measure 1 mm.sup.3. After each tumor quarter has been minced, the particles are plated in culture flasks using sterile pasteur pipettes (9 explants per T-25 or 20 particulates per T-75 flask). Each flask is then labeled with the patient's code, the date of explanation and any other distinguishing data. The explants should be evenly distributed across the bottom surface of the flask, with initial inverted incubation in a 37.degree. C. incubator for 5-10 minutes, followed by addition of about 5-10 ml sterile growth medium and further incubation in the normal, non-inverted position. Flasks are placed in a 35.degree. C., non-CO.sub.2 incubator. Flasks should be checked daily for growth and contamination. Over a period of a few weeks, with weekly removal and replacement of 5 ml of growth medium, the explants will foster growth of cells into a monolayer.

12   With respect to the culturing of malignant cells, it is believed (without any intention of being bound by the theory) that by maintaining the malignant cells within a multicellular particulate of the originating tissue, growth of the malignant cells themselves is facilitated versus the overgrowth of fibroblasts (or other unwanted normal cells) which tends to occur when suspended tumor cells are grown in culture.

13   The use of the above procedure to form a cell monolayer culture maximizes the growth of malignant cells from the tissue sample, and thus optimizes ensuing tissue culture assay of chemotherapeutic action of various agents to be tested. Enhanced growth of actual malignant cells is only one aspect of the present invention; however, another important feature is the growth rate monitoring system used to oversee growth of the monolayer once formed. Once a primary culture and its derived secondary monolayer tissue culture has been initiated, the growth of the cells is monitored to ascertain the time to initiate the chemotherapy assay and to determine the growth rate of the cultured cells.

14   Monitoring of the growth of cells is conducted by counting the cells in the monolayer on a periodic basis, without killing or staining the cells and without removing any cells from the culture flask. The counting may be done visually or by automated methods, either with or without the use of estimating techniques known in the art (counting in a representative area of a grid multiplied by number of grid areas, for example). Data from periodic counting is then used to determine growth rates which may or may not be considered parallel to growth rates of the same cells in vivo in the patient. If growth rate cycles can be documented, for example, then dosing of certain active agents can be customized for the patient. The same growth rate can be used to evaluate radiation treatment periodicity, as well. It should be noted that with the growth rate determinations conducted while the monolayers grow in their flasks, the present method requires no hemocytometry, flow cytometry or use of microscope slides and staining, with all their concomitant labor and cost.

15   Protocols for monolayer growth rate generally use a phase-contrast inverted microscope to examine culture flasks incubated in a 37.degree. C. (5% CO.sub.2) incubator. When the flask is placed under the phase-contrast inverted microscope, ten fields (areas on a grid inherent to the flask) are examined using the 10.times. objective, with the proviso that the ten fields should be non-contiguous, or significantly removed from one another, so that the ten fields are a representative sampling of the whole flask. Percentage cell occupancy for each field examined is noted, and averaging of these percentages

then provides an estimate of overall percent confluency in the cell culture. When patient samples have been divided between two or among three or more flasks, an average cell count for the total patient sample should be calculated. The calculated average percent confluency should be entered into a process log to enable compilation of data--and plotting of growth curves--over time. Monolayer cultures may be photographed to document cell morphology and culture growth patterns.

16   The applicable formula is: ##EQU1##

17   As an example, therefore, if the estimate of area occupied by the cells is 30% and the total area of the field is 100%, percent confluency is 30/100, or 30.

18   Adaptation of the above protocol for non-tumor cells is straightforward and generally constitutes an equivalent procedure.

19   Active agent and/or radiation therapy screening using the cultured cells proceeds with subcultures of the prime culture or, preferably, of the reference culture. The screening can be carried out in an incubation flask, but generally proceeds using plates such as microtiter plates. In a chemotherapy/radiotherapy assay, it is desirable to grow a control culture of a patient's cells in a culture parallel to the reference or prime culture. The control culture can be grown from skin cells, as an easy source of non-malignant cells, from the same organ from which the malignant cells are derived, or from other sources, so long as the cells are typical of non-malignant cells of the patient.

20   The performance of the chemosensitivity/radiosensitivity assay used for screening purposes depends on the ability to deliver a reproducible cell number to each row in a plate and/or a series of plates, as well as the ability to achieve an even distribution of cells throughout a given well. The following procedure assures that cells are reproducibly transferred from flask to microtiter plates, and cells are evenly distributed across the surface of each well.

21   The first step in preparing the microtiter plates is, of course, preparing and monitoring the monolayer as described above. The following protocol is exemplary and susceptible of variation as will be apparent to one skilled in the art. Cells are removed from the culture flask and a cell pellet is prepared by centrifugation. The cell pellet derived from the monolayer is then suspended in 5 ml of the growth medium and mixed in a conical tube with a vortex for 6 to 10 seconds. The tube is then rocked back and forth 10 times. A 36 .mu.l droplet from the center of the conical tube is pipetted onto one well of a 96 well plate. A fresh pipette is then used to pipette a 36 .mu.l aliquot of trypan blue solution, which is added to the same well, and the two droplets are mixed with repeated pipette aspiration. The resulting admixture is then divided between two hemocytometer chambers for examination using a standard light microscope. Cells are counted in two out of four hemocytometer quadrants, under 10.times. magnification. Only those cells which have not taken up the trypan blue dye are counted. This process is repeated for the second counting chamber. An average cell count per chamber is thus determined. Using means known in the art, the quadrant count values are checked, logged, multiplied by 10.sup.4 to give cells/ml, and the total amount of fluid (growth medium) necessary to suspend remaining cell aliquots is calculated accordingly.

22   After the desired concentration of cells in medium has been determined, additional cell aliquots from the monolayer are suspended in growth medium via vortex and rocking and loaded into a Terasaki dispenser known in the art. Aliquots of the prepared cell suspension are delivered into the microtiter plates using Terasaki dispenser techniques known in the art. A plurality of plates may be prepared from a single cell suspension as needed. Plates are then wrapped in sterile wet cotton gauze and incubated in an incubator box by means known in the art.

23   After the microtiter plates have been prepared, exposure of the cells therein

to active agent and/or radiation is conducted according to the following exemplary protocol. During this portion of the inventive assay, the appropriate amount of specific active agent is transferred into the microtiter plates prepared as described above. A general protocol, which may be adapted, follows. Each microtiter plate is unwrapped from its wet cotton gauze sponge and microscopically examined for cell adhesion. Control solution is dispensed into delineated rows of wells within the grid in the microtiter plate, and appropriate aliquots of active agent to be tested are added to the remaining wells in the remaining rows. Ordinarily, sequentially increasing concentrations of the active agent or higher doses of radiation being tested are administered into progressively higher numbered rows in the plate. The plates are then rewrapped in their gauze and incubated in an incubator box at 37.degree. C. under 5% CO.sub.2. After a predefined exposure time, the plates are unwrapped, blotted with sterile gauze to remove the agent, washed with Hank's Balance Salt Solution, flooded with growth medium, and replaced in the incubator in an incubator box for a predefined time period, after which the plates may be fixed and stained for evaluation.

24   Fixing and staining may be conducted according to a number of suitable procedures; the following is representative. After removal of the plates from the incubator box, culture medium is poured off and the plates are flooded with Hank's Balance Salt Solution. After repeated flooding (with agitation each time) the plates are then flooded with reagent grade ethanol for 2-5 minutes. The ethanol is then poured off. Staining is accomplished with approximately 5 ml of Giemsa Stain per plate, although volume is not critical and flooding is the goal. Giemsa stain should be left in place 5 min. .+-.30 seconds as timing influences staining intensity. The Giemsa stain is then poured off and the plates are dipped three times in cold tap water in a beaker. The plates are then inverted, shaken vigorously, and air dried overnight (with plate lids off) on a rack on a laboratory bench. Cells per well are then counted manually or by automated and/or computerized means, to derive data regarding chemosensitivity of cells at various concentrations of exposure. One particularly useful computer operating environment for counting cells is the commercially available OPTIMATE compiler, which is designed to permit an optical counting function well suited to computerized cell counting procedures and subsequent calculations.

25   The above procedures do not change appreciably when cell growth promoters are assayed rather than cell arresting agents such as chemotherapeutic or radiotherapeutic agents. The present assay allows cell death or cell growth to be monitored with equal ease. In any case, optimization of use of the present system will involve the comparative testing of a variety of candidate active agents for selection of the best candidate for patient treatment based upon the in vitro results. One particularly advantageous embodiment of the above described invention comprises a two-stage assay for cytotoxicity followed by evaluation of longer-term inhibitory effect. Chemotherapeutic agents may thus be evaluated separately for both their direct chemotherapeutic effect as well as for their longer duration efficacy.

26   As discussed in brief, above, in parallel with growth of the prime or reference culture, a control culture can be grown. The control culture is a culture of normal cells taken from the same patient from whom the prime culture is collected. The control culture can derive from an epithelial cell sample or can be collected from the same organ as the prime culture so long as the control culture contains no malignant cells. More than one control culture can be maintained. For instance, cultures of both normal skin cells and normal cells of an organ from which the malignancy is derived can be maintained. The value of maintaining a control culture is many fold. Primarily, the control culture serves as a negative control (or positive control, depending upon the marker to be analyzed) in the various analyses to be carried out on the prime culture, the reference culture or subcultures thereof.

27   A second value of the control culture is an indicator of toxicity, the toxicity or undesirable effects of a given therapy upon normal cells. For instance, in

the segregated analysis of chemotherapeutic agents described above, concomitant analysis of the same agents on segregated sites of the control culture would yield an indication of cytotoxicity of the agent with regard to malignant cells versus the toxicity of the agent to control cells. A therapeutic index can be calculated based on the ratio of cytotoxicity to malignant cells to toxicity. Cytotoxicity and toxicity can be quantified as a percentage or fraction of cells killed by a given therapy, or as a percentage or fraction of cells surviving a given therapy. A therapeutic index is a ratio of these percentages or fractions and is reflective of the desirability of a given treatment in a patient. An optimal treatment would be maximally cytotoxic (or even cytostatic) to the malignant cells and minimally toxic to a patient's normal cells.

28 Other indices may be generated, depending upon the desired effect of a therapy. For instance, if a desired therapy is designed to up-regulate a malignancy-specific antigen to promote destruction of the malignancy by a patient's immune system, an index could be generated to discern a treatment which reflects maximal up-regulation of the antigen in the malignant cells and minimal or negative up-regulation in a patient's normal cells. A similar index can be calculated based upon down-regulation of a desired marker (i.e., an angiogenic factor) which can be assayed as either a secreted or a cellular marker and reflects maximal down-regulation of the marker with minimal toxicity or other undesirable effects on the control culture.

29 Often the diseased cells express a cellular marker that is indicative of a certain disease state or lack thereof. However, one aspect of the culture techniques of the present invention is that the cultured diseased cells do not necessarily have to be the cells expressing the factor to be assayed. One question that inevitably arises when considering whether a serum marker is indicative of a particular cancer cell is, which cells produce the marker, the cell or the tissue in which the cancer cells grow? See e.g. Singhal et al., p 610. By co-culturing the cancerous tissue within a multicellular particulate of its originating tissue, the cells (both the diseased cells or the surrounding cells) are better able to retain their production of characteristic markers.

30 Identification of one or more active agents or chemotherapeutic agents is peripheral to the present invention, which is intended for the efficacy screening of any or all of them as to a given patient. Literally any active agent may be screened according to the present invention; listing exemplary active agents is thus omitted here.

31 One important focus of the present invention thus includes the simplicity of the present system--cohesive multicellular particulates of the patient tissue to be tested are used to form cell monolayers; growth of those monolayers is monitored for accurate prediction of correlating growth of the same cells in vivo; and differing concentrations of a number of active agents may be tested for the purpose of determining not only the most appropriate agent but the most appropriate concentration of that agent for actual patient exposure (according to the calculated cell growth rates). It is also important to note, in the context of the invention, that the present system allows in vitro tests to be conducted in suspensions of tissue culture monolayers grown in nutrient medium under fast conditions (a matter of weeks), rather than with single cell progeny produced by dilution cloning over long periods of time. In some cases, the present invention is a two stage assay for both cytotoxicity and the longer-term growth inhibitory.

32 It is additionally possible to increase the value of the assay with the use of staining compositions and protocols designed to characterize the malignant cells thus grown. In other words, the tissue preparation and cell culturing technique itself offers a first assurance that the cells grown out of the tumor are really the malignant tumor cells and not fibroblasts or other nonmalignant cells of no diagnostic value. As a separate confirmation, the present staining compositions and protocols offer a second, independent assurance that the cells subject to diagnostic or prognostic assay are in fact malignant cells in culture. One important characterization has to do with the nature of the

malignant cells as epithelial, which is in turn an indicator of the carcinoma type of malignancy. Other characterizations of malignant cells are intended to fall within the scope of the present invention as well, although the characterization of the cells as epithelial or not is of primary importance.

33   The technique is practiced as follows. The same cell culturing and well distribution process is used as in the cytotoxicity assay described above, but rather than exposing the cells to chemotherapeutic or other agents, the cells are instead fixed and stained. With the stain or stain cocktail described below, the epithelial cells are identified by their intermediate filaments and/or specific membrane antigens by means of a monoclonal antibody immunoperoxidase technique. The fixative used can be any fixative which does not alter the cellular molecular markers of interest. The fixed, stained cells are then counted. If the specimen is positive for epithelial cells, the process is complete. If the specimen is negative for epithelial cells, an independent fixing and staining process is subsequently completed, with fresh cells from identical wells, using Vimentin as a stain to confirm the non-epithelial nature of the cells.

34   The importance of having a stain or stain cocktail (i.e., antibody cocktail), as well as an overall protocol, for identifying epithelial cells in biopsies of malignant tumors is as follows. In the basic cytotoxicity assay, the tissue culture technique is designed to grow out the cells of the tumor of origin and in fact consistently does so. Despite such reliable predictability, however, the fact that the cells of the tumor of origin did in fact grow out, and not fibroblasts or other cells, must be confirmed with independent proof before the cells can be used with complete assurance in the appropriate patient assay(s). The present technology provides a means to obtain this confirmation, which in turn furthers the interests of good laboratory and medical practice.

35   As a general consideration, the staining compounds or compositions of interest for use in the present technology are those which bind with cellular molecular markers unique either to epithelial or to non-epithelial cells. A further aspect of the invention therefore inheres in the following two aspects: the improvement of the cytotoxicity assay by adding the epithelial staining protocol with any known epithelial stain; and the further improvement wherein specially designed stain cocktails maximize the likelihood that the presence of any known intermediate filament or specific membrane antigen, characteristic of epithelial cells, will be identified if present.

36   Many carcinomas are positive for any one of the intermediate filaments or specific membrane antigens characteristic of epithelial cells; virtually all if not all carcinomas are positive for one of a number of such intermediate filaments or specific membrane antigens. For example, "epithelial membrane antigen" (EMA) glycoproteins are known in the art and can be bound with various antiepithelial membrane antigen antibodies including monoclonal antibodies. Cytokeratin is another important epithelial cell marker and binding reagents including monoclonal antibodies are available which are specific to cytokeratin. While antisera can be raised in vivo against markers such as EMA glycoproteins and cytokeratin, as a practical matter commercially available polyclonal or monoclonal antibodies are used in the following protocols, with monoclonal antibodies being preferred.

37   Binding of the epithelial marker is revealed with associated staining procedures and reactions which give a visual indication that the marker binding took place. Those skilled in the art already appreciate various techniques already available--in the general field of "immunocytochemistry"--to reveal antibody-antigen re-actions. One known way to accomplish this visualization when antibody binding reagents are used is with the "labeled streptavidin procedure". In this procedure, after the specimen is exposed to antibodies specific to the target antigen, a secondary "link" antibody is added. The secondary biotinylated "link" antibody consists of anti-mouse and anti-rabbit antibodies which bind universally to most primary monoclonal or polyclonal antibodies. The "link" will also connect to the tertiary reagent

.(peroxidase-labeled streptavidin) through chemical bonding between the biotin on the secondary reagent and the streptavidin on the streptavidin/peroxidase conjugate. Staining is completed by incubating the specimen and primary, secondary and tertiary agents in the presence of a chromogen, so that the peroxidase and the chromogen form a visible precipitate. Alternatively, a fluorescein-based detection system can be used to visualize the primary antibody, or a third alternative known in the art as the digoxigenin-conjugated detection system may be used.

38   Of the various epithelial markers, three have received the most widespread attention in the literature: EMA glycoproteins, cytokeratin, and carcinoembryonic antigen. In the context of this invention, the first two are the most important because literally any epithelial cell will have at least either one EMA glycoprotein on the surface thereof or a cytokeratin intermediate filament present. Therefore, the present invention resides not only in binding and staining for an epithelial marker on the surfaces of the specimen cells, but in simultaneously assaying for either or both of EMA glycoprotein(s) and cytokeratin. The cocktails of the present invention therefore contain binding reagents for both EMA glycoproteins and cytokeratin and, importantly, are selected to include the most generally applicable binding reagents in combination so that the cocktail has the broadest binding scope possible. The cocktails identified in Examples 1 and 2, for example, represent a combination of two general binding reagents (containing a total of three monoclonal antibodies) for cytokeratin, admixed with a general binding reagent for EMA glycoprotein. The dual benefit of this admixture of general binding agents is that the incidence of false negatives for epithelial cells is minimized, and the visible staining reactions are generally stronger when the combined binding reagents are used in lieu of a single binding reagent.

39   Although the binding reagents and other reagents identified in the Examples are the preferred reagents for use in the context of the invention, the invention is intended to encompass epithelial-specific binding and staining reagents generally. These include, without limitation: Boehringer-Mannheim AE1 anti-cytokeratin antibody; Boehringer-Mannheim AE3 anti-cytokeratin antibody; Boehringer-Mannheim AE1/AE3 anti-cytokeratin antibody (AE1 and AE3 in admixture); Becton-Dickinson CAM 5.2 antibody, DAKO EMA antibody, Biomeda's Anti-Cytokeratin Cocktail CK22, Biomeda's Anti-Cytokeratin Cocktail CK23, Biomeda's Anti-Pan-Cytokeratin CK56, Biomeda's polyclonal goat or rabbit anti-cytokeratin antisera, ScyTek Laboratories' anti-EMA antigen antibody clone E29, and many others. Those skilled in the art and in possession of the guidance provided herein can readily determine alternative, equivalent binding and staining reagents and cocktails, to accomplish the disclosed result. These binding agents and cocktails may be used in combination with any known visualization system, such as the streptavidin, fluorescein- and digoxigenin-conjugated systems identified above.

40   As a control, Vimentin antibody is used as a binding alternative either in conjunction with binding and staining of the test cells, or subsequently thereto. In the context of this invention, Vimentin can be considered a binding reagent which is specific to non-epithelial cells of mesenchymal origin.

41   In a further aspect of the present invention, immunological markers may be monitored in applications requiring up- or down-regulation of such markers (i.e., Major histocompatibility complex molecules). This aspect of the present invention can be especially useful in transplantation applications where, for instance, through chemical or biological means rejection of transplanted cells is sought to be avoided by down-regulation of the various transplantation antigens present on the cells to be transplanted. The present invention would be especially useful in monitoring such immunoregulation.

42   Lastly, cell morphology can be assayed by culturing cells of, i.e., the prime culture or the reference culture, removing the cells from the surface upon which they grow, centrifuging cells into a loose pellet and growing the cell pellet over a defined time period. By growing cells in this manner, it is

possible to view the cohesive morphology of cells in a cluster resembling a tumor.

43   EXAMPLE 1

44   Radiation Therapy

45   Separate 50 mg samples from residual tissue from specimens of three human glioblastomas and one human ovarian carcinoma were minced in medium with sterile scissors to a particle size of roughly 1 mm.sup.3 and with a particle size distribution between about 0.25 and about 1.5 mm.sup.3. The medium was Standard F-10 medium containing 17% calf serum and a standard amount of Penicillin and Streptomycin. Each 50 mg sample was minced and was divided into four groups of particulates and each of 16 groups was charged to a separate labeled culture flask containing the above-described medium. Visual confirmation was made that the particulates were evenly distributed along the bottom of each flask and the flasks were placed in a 35.degree. C., non-CO.sub.2 incubator. Flasks were checked daily for growth and contamination. Over a period of a few weeks, with weekly removal and replacement of 5 ml of growth medium, the particulates grew into monolayers.

46   Enough cells were then removed from the monolayers grown in the flasks for centrifugation into standard size cell pellets for each of the 16 flasks. Each cell pellet was then suspended in 5 ml of the above-described medium and was mixed in a conical tube with a vortex for 6 to 10 seconds, followed by manual rocking back and forth 10 times. A 36 ml droplet from the center of each tube was then pipetted into one well of a 96-well microtiter plate together with an equal amount of trypan blue, plus stirring. The resulting admixture was then divided between two hemocytometer quadrants for examination using a standard light microscope. Cells were counted in two out of four hemocytometer quadrants, under 10.times. magnification--only those cells which had not taken up the trypan blue dye were counted. This process was repeated for the second counting chamber. An average cell count per chamber was calculated and by means known in the art the optimum concentration of cells in the medium was determined.

47   Accommodating the above calculations, additional cell aliquots from the 16 monolayers were separately suspended in growth medium via vortex and rocking and were loaded into a Terasaki dispenser adapted to a 60-well plate. Aliquots of the prepared cell suspension were delivered into the microtiter plates using Terasaki dispenser techniques known in the art. Cells were plated into 60-well microtiter plates at a concentration of 100 cells per well.

48   Twenty-four (24) hours later, the cells were irradiated using a Siemens Stabilipan X-ray machine at 250 kVp, 15 mA with a dose rate of 75 rad/minute. For each radiation dose from 1 Gy to 6 Gy, cell number per well was monitored as a function of time through five days post-irradiation.

49   Cell number relative to controls was determined and survival curves were fit to the data. The rate of decrease in survival as a function of time was proportional to dose. A differential radiation response among the four cell lines was observed.

50   EXAMPLE 2

51   Immuno Therapy

52   Separate 50 mg samples from residual tissue from specimens of a human brain tumor, renal carcinoma, and breast carcinoma were minced in medium with sterile scissors to a particle size of roughly 1 mm.sup.3 and with a particle size distribution between about 0.25 and about 1.5 mm.sup.3. The medium was Standard F-10 medium containing 17% calf serum and a standard amount of Penicillin and Streptomycin. Each 50 mg sample was minced and was divided into four groups of particulates and each of 12 groups was charged to a separate labeled culture

flask containing the above-described medium. Visual confirmation was made that the particulates were evenly distributed along the bottom of each flask and the flasks were placed in a 35.degree. C., non-CO.sub.2 incubator. Flasks were checked daily for growth and contamination. Over a period of a few weeks, with weekly removal and replacement of 5 ml of growth medium, the particulates grew into monolayers.

53   Enough cells were then removed from the monolayers grown in the flasks for centrifugation into standard size cell pellets for each of the twelve flasks. Each cell pellet was then suspended in 5 ml of the above-described medium and was mixed in a conical tube with a vortex for 6 to 10 seconds, followed by manual rocking back and forth 10 times. A 36 ml droplet from the center of each tube was then pipetted into one well a 96-well microtiter plate together with an equal amount of trypan blue, plus stirring. The resulting admixture was then divided between two hemocytometer quadrants for examination using a standard light microscope. Cells were counted in two out of four hemocytometer quadrants, under 10.times. magnification--only those cells which had not taken up the trypan blue dye were counted. This process was repeated for the second counting chamber. An average cell count per chamber was calculated and by means known in the art the optimum concentration of cells in the medium was determined.

54   Accommodating the above calculations, additional cell aliquots from the 12 monolayers were separately suspended in growth medium via vortex and rocking and were loaded into a Terasaki dispenser adapted to a 60-well plate. Aliquots of the prepared cell suspension were delivered into the microtiter plates using Terasaki dispenser techniques known in the art. Cells were plated into 60-well microtiter plates at a concentration of 100 cells per well.

55   Twenty-four (24) hours post-plating, Activated Natural Killer (ANK) cells were delivered into a row of six wells by means of a micropipette. In each microtiter plate three rows of six wells each served as controls. The effector (ANK cells):target cell (tumor cells) ratio varied from 2.5:1 to 20:1. The ANK cells were exposed to the target cells for four hours. Subsequently, the wells were washed with Hanks Balanced Salt Solution and the number of ANK cells remaining in the wells was observed with a phase contrast microscope. This process was repeated until no ANK cells remained in the wells (usually 3 washes). Following removal of the ANK cells, the tumor cells were incubated in the wells for another 24 hours.

56   Cell number relative to control was determined. For the three tumor types increasing the effector:target cell ratio from 2.5:1 to 20:1 resulted in an increase in the number of tumor cells killed by the ANK cells.

57   EXAMPLE 3

58   Gene Therapy/Antisense Oligonucleotides

59   A 50 mg sample from a residual human mesothelioma was minced in medium with sterile scissors to a particle size of roughly 1 mm.sup.3 and with a particle size distribution between about 0.25 and about 1.5 mm.sup.3. The medium was Standard F-10 medium containing 17% calf serum and a standard amount of Penicillin and Streptomycin. The 50 mg sample was minced and was divided into four groups of particulates and each of four groups was charged to a separate labeled culture flask containing the above-described medium. Visual confirmation was made that the particulates were evenly distributed along the bottom of each flask and the flasks were placed in a 35.degree. C., non-CO.sub.2 incubator. Flasks were checked daily for growth and contamination. Over a period of a few weeks, with weekly removal and replacement of 5 ml of growth medium, the particulates grew into monolayers.

60   Enough cells were then removed from the monolayers grown in the flasks for centrifugation into standard size cell pellets for each of the four flasks. Each cell pellet was then suspended in 5 ml of the above-described medium and

was mixed in a conical tube with a vortex for 6 to 10 seconds, followed by manual rocking back and forth 10 times. A 36 ml droplet from the center of each tube was then pipetted into one well of a 96-well microtiter plate together with an equal amount of trypan blue, plus stirring. The resulting admixture was then divided between two hemocytometer quadrants for examination using a standard light microscope. Cells were counted in two out of four hemocytometer quadrants, under 10.times. magnification--only those cells which had not taken up the trypan blue dye were counted. This process was repeated for the second counting chamber. An average cell count per chamber was calculated and by means known in the art the optimum concentration of cells in the medium was determined.

61 Accommodating the above calculations, additional cell aliquots from the 4 monolayers were separately suspended in growth medium via vortex and rocking and were loaded into a Terasaki dispenser adapted to a 60-well plate. Aliquots of the prepared cell suspension were delivered into the microtiter plates using Terasaki dispenser techniques known in the art. Cells were plated into 60-well microtiter plates at a concentration of 100 cells per well.

62 Twenty-four (24) hours post-plating, antisense oligonucleotide for the urokinase-type plasminogen activator receptor (uPAR) was delivered to wells in the microtiter plate. Proteolysis of plasminogen to plasmin by urokinase-type plasminogen activator has been implicated in the processes of tumor cell proliferation and invasion. The concentrations of the uPAR antisense oligonucleotide were 1, 10 and 100 micromolar. uPAR sense and missense oligonucleotides at the concentrations of 1, 10 and 100 micromolar served as controls. The tumor cells were exposed to the oligonucleotides for 24 hours and then the agents were removed. The cells were allowed to incubate for another 72 hours so that inhibition of cell proliferation could be observed.

63 Cell number relative to control was then determined. Antisense oligbnucleotides to uPAR suppressed the proliferative activity of the tumor cells in a concentration dependent manner.

64 EXAMPLE 4

65 Combination Chemotherapy

66 Separate 50 mg samples from residual tissue from specimens from four human ovarian tumors were minced in medium with sterile scissors to a particle size of roughly 1 mm.sup.3 and with a particle size distribution between about 0.25 and about 1.5 mm.sup.3. The medium was Standard F-10 medium containing 17% calf serum and a standard amount of Penicillin and Streptomycin. Each 50 mg sample was minced and was divided into 4 groups of particulates and each of 16 groups was charged to a separate labeled culture flask containing the above-described medium. Visual confirmation was made that the particulates were evenly distributed along the bottom of each flask and the flasks were placed in a 35.degree. C., non-CO.sub.2 incubator. Flasks were checked daily for growth and contamination. Over a period of a few weeks, with weekly removal and replacement of 5 ml of growth medium, the particulates grew into monolayers.

67 Enough cells were then removed from the monolayers grown in the flasks for centrifugation into standard size cell pellets for each of the 16 flasks. Each cell pellet was then suspended in 5 ml of the above-described medium and was mixed in a conical tube with a vortex for 6 to 10 seconds, followed by manual rocking back and forth 10 times. A 36 ml droplet from the center of each tube was then pipetted into one well of a 96-well microtiter plate together with an equal amount of trypan blue, plus stirring. The resulting admixture was then divided between two hemocytometer quadrants for examination using a standard light microscope. Cells were counted in two out of four hemocytometer quadrants, under 10.times. magnification--only those cells which had not taken up the trypan blue dye were counted. This process was repeated for the second counting chamber. An average cell count per chamber was calculated and by means known in the art the optimum concentration of cells in the medium was

determined.

68　Accommodating the above calculations, additional cell aliquots from the 16 monolayers were separately suspended in growth medium via vortex and rocking and were loaded into a Terasaki dispenser adapted to a 60-well plate. Aliquots of the prepared cell suspension were delivered into the microtiter plates using Terasaki dispenser techniques known in the art. Cells were plated into 60-well microtiter plates at a concentration of 100 cells per well.

69　Twenty-four (24) hours post-plating, the chemotherapeutic agent Taxol was applied to the wells in the microtiter plates. The first three treatment rows in the plates (Rows 2, 3, and 4) were designed to have escalating Taxol doses (1.0, 5.0, and 25 .mu.M) with a fixed carboplatin dose (200 .mu.M). The last three treatment rows in the plates (Rows 6, 7, and 9) were designed to have a fixed Taxol dose (5 .mu.M) with an escalating carboplatin dose (50, 200, and 1000 .mu.M). Rows 5 and 9 served as a control. The Taxol exposure time was two hours. Twenty-four hours later, the cells in the wells were exposed to carboplatin for two hours. The tumor cells in the wells were then incubated for another 48 hours.

70　Cell number relative to control was determined. For the cells from the four tumor specimens a dose response relationship was observed for both the escalating Taxol/fixed carboplatin and fixed Taxol/escalating carboplatin treatment schema.

71　EXAMPLE 5

72　Hormonal Therapy

73　Separate 50 mg samples from residual tissue from specimens from four human breast tumors were minced in medium with sterile scissors to a particle size of roughly 1 mm.sup.3 and with a particle size distribution between about 0.25 and about 1.5 mm . The medium was Standard F-10 medium containing 17% calf serum and a standard amount of Penicillin and Streptomycin. Each 50 mg sample was minced and was divided into four groups of particulates and each of 16 groups was charged to a separate labeled culture flask containing the above-described medium. Visual confirmation was made that the particulates were evenly distributed along the bottom of each flask and the flasks were placed in a 35.degree. C., non-CO.sub.2 incubator. Flasks were checked daily for growth and contamination. Over a period of a few weeks, with weekly removal and replacement of 5 ml of growth medium, the particulates grew into monolayers.

74　Enough cells were then removed from the monolayers grown in the flasks for centrifugation into standard size cell pellets for each of the 16 flasks. Each cell pellet was then suspended in 5 ml of the above-described medium and was mixed in a conical tube with a vortex for 6 to 10 seconds, followed by manual rocking back and forth 10 times. A 36 ml droplet from the center of each tube was then pipetted into one well of a 96-well microtiter plate together with an equal amount of trypan blue, plus stirring. The resulting admixture was then divided between two hemocytometer quadrants for examination using a standard light microscope. Cells were counted in two out of four hemocytometer quadrants, under 10.times. magnification--only those cells which had not taken up the trypan blue dye were counted. This process was repeated for the second counting chamber. An average cell count per chamber was calculated and by means known in the art the optimum concentration of cells in the medium was determined.

75　Accommodating the above calculations, additional cell aliquots from the 16 monolayers were separately suspended in growth medium via vortex and rocking and were loaded into a Terasaki dispenser adapted to a 60-well plate. Aliquots of the prepared cell suspension were delivered into the microtiter plates using Terasaki dispenser techniques known in the art. Cells were plated into 60-well microtiter plates at a concentration of 100 cells per well.

76   Twenty-four (24) hours post-plating, the antiestrogenic compound Tamoxifen was
      delivered to wells in the microtiter plates. A stock solution of Tamoxifen was
      initially prepared by dissolving 1.5 mg of Tamoxifen powder in 1 ml of absolute
      ethanol and then adding 9 ml of growth medium. This stock solution was then
      used to make Tamoxifen solutions in the concentration range of 10 nM to 20
      .mu.M. Six doses of Tamoxifen were used for cells from each of the four breast
      tumor specimens. An ethanol solution at a concentration equivalent to that at
      the highest Tamoxifen concentration served as a control. The tumor cells were
      exposed to Tamoxifen for 24 hours and then the agent was removed. The cells
      were allowed to incubate for another 72 hours so that inhibition of cell
      proliferation could be observed.

77   Cell number relative to control was then determined. There was no effect
      observed when the ethanol-only control wells were compared to the growth
      medium-only control wells. The cells of two of the four breast specimens tested
      showed an inhibition of cell proliferation by Tamoxifen exposure. These
      responses occurred in the mid to high Tamoxifen concentration ranges.

78   EXAMPLE 6

79   Differentiating Agent Therapy ("Biological Response Modification")

80   Separate 50 mg samples from residual tissue from specimens from four human
      breast tumors were minced in medium with sterile scissors to a particle size of
      roughly 1 mm.sup.3 and with a particle size distribution between about 0.25 and
      about 1.5 mm.sup.3. The medium was Standard F-10 medium containing 17% calf
      serum and a standard amount of Penicillin and Streptomycin. Each 50 mg sample
      was minced and was divided into four groups of particulates and each of 16
      groups was charged to a separate labeled culture flask containing the
      above-described medium. Visual confirmation was made that the particulates were
      evenly distributed along the bottom of each flask and the flasks were placed in
      a 35.degree. C., non-CO.sub.2 incubator. Flasks were checked daily for growth
      and contamination. Over a period of a few weeks, with weekly removal and
      replacement of 5 ml of growth medium, the particulates grew into monolayers.

81   Enough cells were then removed from the monolayers grown in the flasks for
      centrifugation into standard size cell pellets for each of the 16 flasks. Each
      cell pellet was then suspended in 5 ml of the above-described medium and was
      mixed in a conical tube with a vortex for 6 to 10 seconds, followed by manual
      rocking back and forth 10 times. A 36 ml droplet from the center of each tube
      was then pipetted into one well of a 96-well microtiter plate together with an
      equal amount of trypan blue, plus stirring. The resulting admixture was then
      divided between two hemocytometer quadrants for examination using a standard
      light microscope. Cells were counted in two out of four hemocytometer
      quadrants, under 10.times. magnification--only those cells which had not taken
      up the trypan blue dye were counted. This process was repeated for the second
      counting chamber. An average cell count per chamber was calculated and by means
      known in the art the optimum concentration of cells in the medium was
      determined.

82   Accommodating the above calculations, additional cell aliquots from the 16
      monolayers were separately suspended in growth medium via vortex and rocking
      and were loaded into a Terasaki dispenser adapted to a 60-well plate. Aliquots
      of the prepared cell suspension were delivered into the microtiter plates using
      Terasaki dispenser techniques known in the art. Cells were plated into 60-well
      microtiter plates at a concentration of 100 cells per well.

83   Twenty-four (24) hours post-plating the differentiating agent retinoic acid was
      delivered to wells in the microtiter plates. A stock solution of retinoic acid
      was initially prepared by dissolving retinoic acid powder in 1 ml of dimethyl
      sulfoxide (DMSO) and then adding 9 ml of growth medium. This stock solution was
      then used to make retinoic acid solutions in the concentration range of 0.1 to
      1.0 mM. Six doses of retinoic acid were used for cells from each of the four
      breast tumor specimens. A DMSO solution at a concentration equivalent to that

at the highest retinoic acid concentration served as a control. The tumor cells were exposed to retinoic acid for 24 hours and then the agent was removed. The cells were allowed to incubate for another 72 hours so that inhibition of cell proliferation could be observed.

84   Cell number relative to control was then determined. There was no effect observed when the DMSO-only control wells were compared to the growth medium-only control wells. The cells of three of the four breast specimens tested showed an inhibition of cell proliferation by retinoic acid exposure. These responses occurred in the mid to high retinoic acid concentration ranges.

85   EXAMPLE 7

86   Combined Modality Therapy Drug/Radiation

87   Separate 50 mg samples from residual tissue from specimens from two human brain tumors and two human ovarian tumors were minced in medium with sterile scissors to a particle size of roughly 1 mm.sup.3 and with a particle size distribution between about 0.25 and about 1.5 mm.sup.3. The medium was Standard F-10 medium containing 17% calf serum and a standard amount of Penicillin and Streptomycin. Each 50 mg sample was minced and was divided into four groups of particulates and each of 16 groups was charged to a separate labeled culture flask containing the above-described medium. Visual confirmation was made that the particulates were evenly distributed along the bottom of each flask and the flasks were placed in a 35.degree. C., non-CO.sub.2 incubator. Flasks were checked daily for growth and contamination. Over a period of a few weeks, with weekly removal and replacement of 5 ml of growth medium, the particulates grew into monolayers.

88   Enough cells were then removed from the monolayers grown in the flasks for centrifugation into standard size cell pellets for each of the 16 flasks. Each cell pellet was then suspended in 5 ml of the above-described medium and was mixed in a conical tube with a vortex for 6 to 10 seconds, followed by manual rocking back and forth 10 times. A 36 ml droplet from the center of each tube was then pipetted into one well of a 96-well microtiter plate together with an equal amount of trypan blue, plus stirring. The resulting admixture was then divided between two hemocytometer quadrants for examination using a standard light microscope. Cells were counted in two out of four hemocytometer quadrants, under 10.times. magnification--only those cells which had not taken up the trypan blue dye were counted. This process was repeated for the second counting chamber. An average cell count per chamber was calculated and by means known in the art the optimum concentration of cells in the medium was determined.

89   Accommodating the above calculations, additional cell aliquots from the 16 monolayers were separately suspended in growth medium via vortex and rocking and were loaded into a Terasaki dispenser adapted to a 60-well plate. Aliquots of the prepared cell suspension were delivered into the microtiter plates using Terasaki dispenser techniques known in the art. Cells were plated into 60-well microtiter plates at a concentration of 100 cells per well.

90   Twenty-four (24) hours post-plating, cells in the microtiter plate wells were exposed to the chemotherapeutic agent Taxol. One set of plates was designed to have escalating Taxol doses with (0.5-25.0 .mu.M) with a fixed radiation dose (2 Gy). A second set of plates was designed to have a fixed Taxol dose (5 .mu.M) with an escalating radiation dose (1 Gy-6 Gy). The cells in the plates were irradiated using a Siemans Stabilipan X-ray machine operating at 250 kVp, 15 mA with a dose rate of 75 rad/minute.

91   For each of the two treatment schema, cell number per well was monitored as a function of time through 5 days post-treatment. Cell number relative to controls was determined and survival curves were fit. A differential response among the cells from the four tumor specimens was observed. Both additive and synergistic cell killing was noted.

92   EXAMPLE 8

93   Initiation of a Prime Culture

94   A tumor biopsy of approximately 100 mg of non-necrotic, non-contaminated tissue
      was harvested from the patient by surgical biopsy and transferred to the
      laboratory in a standard shipping container. Biopsy sample preparation
      proceeded as follows. Reagent grade ethanol was used to wipe down the surface
      of a Laminar flow hood. The tumor was then removed, under sterile conditions,
      from its shipping container, and cut into quarters with a sterile scalpel.
      Using sterile forceps, each undivided tissue quarter was then placed in 3 ml
      sterile growth medium (Standard F-10 medium containing 17% calf serum and a
      standard amount of Penicillin and Streptomycin) and was systematically minced
      by using two sterile scalpels in a scissor-like motion. The tumor particulates
      each measured about 1 mm.sup.3. After each tumor quarter was minced, the
      particles were plated in culture flasks using sterile pasteur pipettes (9
      explants per T-25 or 20 particulates per T-75 flask). Each flask was then
      labeled with the patient's code, the date of explanation and any other
      distinguishing data. The explants were evenly distributed across the bottom
      surface of the flask, with initial inverted incubation in a 37.degree. C.
      incubator for 5-10 minutes, followed by addition of about 5-10 ml sterile
      growth medium and further incubation in the - normal, non-inverted position.
      Flasks were placed in a 35.degree. C., non-CO.sub.2 incubator. Flasks were
      checked daily for growth and contamination. Over a period of a few weeks, with
      weekly removal and replacement of 5 ml of growth medium, the explants grew out
      into a monolayer.

95   EXAMPLE 9

96   Unified Tracking System

97   a. Growth Rate

98   Following initiation of prime cell culture of a tumor specimen, the growth rate
      of the cells was determined until the chemosensitivity assay was performed.
      During this time period the growth was monitored by observing the percent of
      confluency of the cells in a flask. These data provide information valuable as
      a correlation to possible growth of the tumor in the patient as well as for the
      interpretation of the results of the chemosensitivity assay.

99   Three examples of growth rate data are shown in FIGS. 1A-1C. The percent of
      confluency of the cultured cells is plotted as a function of time after the
      initial seeding of the tissue specimen.

100   Slow Growth Rate (FIG. 1A): 25% confluent after 19 days Moderate Growth Rate
      (FIG. 1B): 60% confluent after 21 days Fast Growth Rate (FIG. 1C): 90%
      confluent after 11 days

101   b. Immunohistochemical Staining for Cell Characterization, etc.

102   Many tumor specimens will contain a mixture of cancer and normal cells.
      Although in many cases tumor cells will readily grow in tissue culture, while
      the normal cells will not, it is important to be able to distinguish the two
      cell types. Using immunoperoxidase techniques to stain cells for various
      intermediate filaments, the differences between normal (fibroblast-like) cells
      and cells from epithelial tumors were characterized. These techniques can also
      be used to identify other tumor cell characteristics which may have prognostic
      value.

103   An initial attempt at cultured cell characterization has been to use known
      epithelial tumor cell lines and a known fibroblast cell line. The epithelial
      tumor cell lines all have stained positively for a mixture ("cocktail") of

epithelial intermediate filament antibodies, (not every line, however, has stained positively for the three antibodies within the mixture [AE1/AE3; Cam 5.2; EMA]). Some of the epithelial tumor cells in culture also stained mildly positive for an antibody against an intermediate filament characteristic of fibroblasts (vimentin). When staining for fibroblast intermediate filament (vimentin) in cell culture, all fibroblast cells were positive. Some focal staining by epithelial tumor cells for vimentin was also present..

	epithelial cocktail	vimentin
epithelial tumor cells	++	+
fibroblasts	–	++

104   Testing of intermediate filaments with antibodies for epithelial cells and vimentin appears to be a method of distinguishing certain characteristics of tumor and normal cells.

105   c. Response to Chemotherapy

106   The tissue culture chemosensitivity assay has been refined to make it more sensitive for the detection of damage produced by a variety of chemotherapeutic agents. The initial alteration was to allow a 24-hour time period between plating of cells in microtiter wells and the exposure to drugs. This time interval permits cells to be in an active state of proliferation, where they are more sensitive to cell cycle active agents. The second change was to initiate a long-term assay (growth inhibition assay) over a period of about 72 hours. The short-term assay is conducted 24-72 hours after the therapeutic agent is added. The longer time between drug exposure and assay allows for the detection of cell damage which occurs over a protracted period and requires several cell division cycles before it becomes apparent. "CI" is a measure of the relative survival rates of a given cell culture. It is calculated by according to the formula: ##EQU2##

107   The data for a short-term assay and a long-term assay performed on two sets of patient cultured cells are presented in FIGS. 2A-2F through 5A-5F. The long-term assay (FIGS. 3A-3F and 5A-5F) may both accentuate a positive result obtained from the short-term assay (FIGS. 2A-2F and 4A-4F) and reveal an effect not observed during the short-term assay. The long-term assay is now incorporated into the tissue culture chemosensitivity on a routine basis.

108   d. Response to Radiation Therapy

109   The use of the microtiter well assay to analyze the direct effect of radiation therapy on tumor cells in culture has resulted in a rapid evaluation method for the determination of inherent cellular radiation response. As an example, two radiation dose-response curves generated from the microtiter well assay are presented in FIGS. 6 and 7. The cells from the tumor specimen in FIG. 6 are more resistant than those of the specimen in FIG. 7. The more resistant tumor has been previously irradiated.

110   The microtiter well assay is ideally suited for examination of the interaction of chemotherapeutic agents and radiation. Issues such as the differential sensitivity of drug/radiation combinations and the timing of drug/radiation combinations may be directly addressed with this system. An illustration of chemotherapeutic agent enhancement of radiation response is presented in FIGS. 8A-8C.

111   FIG. 8A: Radiation-only at 2 Gy and 4 Gy FIG. 8B: Taxol 8.5 ng/ml+2 Gy and 4 Gy

FIG. 8C: Taxol 42.5 ng/ml+2 Gy and 4 Gy

112   e. Response to Cellular Immunotherapy

113   Activated lymphocytes are being used as a treatment for some types of cancer.
      These Activated Natural Killers (ANK) cells have been shown to mediate highly
      efficient cell killing for some tumor types. The microtiter well assay can be
      utilized to make a rapid assessment of ANK-induced tumor target cell killing.
      An illustration of two such interactions is presented in FIGS. 9A and 9B.

114   In FIGS. 9A and 9B, the target cells were from a melanoma and a renal
      carcinoma, respectively. The target cells were exposed to the ANK cells for 4
      hours and then the assay was performed. The effector:target cell ratio varied
      from 1:20 to 1:2.5. The data show increasing cell killing as a function of
      increasing effector:target ratio.

115   f. Use of Tissue Culture Medium for Determination of Factors with Possible
      Prognostic/Biological Significance

116   A number of substances secreted by tumor cells such as Tumor Associated
      Antigens and Plasminogen Activators and Inhibitors are believed to regulate a
      variety of processes involved in the progression of malignant disease. Many of
      these factors are produced by tumor cells growing in tissue culture and are
      secreted into the growth medium. The measurement of these factors in the medium
      from cell cultures of tumor specimens may prove to be of predictive value in
      the assessment of the biological behavior of individual cancers.

117   Preliminary work in this area has been on the detection of plasminogen
      activator inhibitor in the growth medium of glioblastoma cell lines.
      Plasminogen activator inhibitor expression has been shown to be increased in
      malignant brain tumors in patients. Medium from glioblastoma cell lines showed
      an increase in plasminogen activator inhibitor when compared to the medium
      alone.

118   Any or all of the steps of the unified assays and culturing techniques of the
      present invention may be automated. Indices can be automatically calculated by
      a computer which is programmed appropriately. Data can be input into the
      computer either manually or automatically, into a spreadsheet or database
      program, or the like. The spreadsheet or database program can be programed to
      reduce the data to the indices described above, or to any other relevant form,
      i.e., graphical or figurative representations of the data.

119   In one example, the cells to be assayed are grown on microtiter plates and
      assayed for their sensitivity to a chemotherapeutic agent according to the
      above-described protocols. The microtiter plates are read on an optical scanner
      and data from the scanner is automatically exported to a computer for
      calculation of a therapeutic index. Other types of scanners may be utilized
      depending upon the assay. For instance, a scanner for reading RIA data would be
      provided if the assay is an RIA assay.

120   Although the present invention has been described with respect to specific
      materials and methods above, the invention is only to be considered limited
      insofar as is set forth in the accompanying claims.

CLAIMS:

I claim:

1. A method of observing the behavior of cells derived from a sample of cancer
cells, comprising:

(a) collecting a specimen of a patient's malignant or hyperproliferative cells;

(b) mechanically separating said specimen into cohesive multicellular particulates having a particle size between 0.25-1.5 mm.sup.3 ;

(c) growing a tissue culture monolayer from said multicellular particulates to form a prime culture which may be maintained;

(d) forming at least one subculture of said prime culture for further analysis; and

(e) monitoring said prime culture over a period of time for its characteristics to observe the behavior of the cells in said prime culture.

2. A method of observing the behavior of cells derived from a sample of cancer cells as claimed in claim 1, further comprising the step of maintaining the prime culture.

3. A method of observing the behavior of cells derived from a sample of cancer cells as claimed in claim 1, further comprising the steps of preparing a reference culture from the prime culture and treating the reference culture with one or more treatments as given to the patient.

4. A method of observing the behavior of cells derived from a sample of cancer cells as claimed in claim 3, further comprising the steps of preparing a subculture of one of the prime culture and the reference culture.

5. A method of observing the behavior of cells derived from a sample of cancer cells as claimed in claim 4, further comprising the step of assaying for a malignancy-specific marker in one of the prime culture, the reference culture, the subculture and tissue culture medium used to grow one of the prime culture, the reference culture or the subculture.

6. A method of observing the behavior of cells derived from a sample of cancer cells as claimed in claim 5, wherein the marker indicates one of aggressiveness and invasiveness of the cells in said prime culture.

7. A method of observing the behavior of cells derived from a sample of cancer cells as claimed in claim 6, wherein the marker is vascular endothelial growth factor.

8. A method of observing the behavior of cells derived from a sample of cancer cells as claimed in claim 5, wherein the marker is indicative of complications associated with the cells in said prime culture.

9. A method of observing the behavior of cells derived from a sample of cancer cells as claimed in claim 8, wherein the marker is indicative of a thrombogenic potential.

10. A method of observing the behavior of cells derived from a sample of cancer cells as claimed in claim 5, wherein the marker is identified by one of cytochemistry or immunohistochemistry.

11. A method of observing the behavior of cells derived from a sample of cancer cells as claimed in claim 10, wherein the marker is selected from the group consisting of an estrogen receptor, a progesterone receptor, an oncogene, a product of an oncogene, a marker for multi-drug resistance and a marker for phenotypic characterization.

12. A method of observing the behavior of cells derived from a sample of cancer cells as claimed in claim 5, wherein one or more of the steps are at least partially automated.

13. A method of observing the behavior of cells derived from a sample of cancer cells as claimed in claim 5, wherein the marker is characterized by a molecular

biological technique.

14. A method of observing the behavior of cells derived from a sample of cancer cells as claimed in claim 13, wherein the molecular biological technique characterizes one of tumor cell heterogeneity or specific mutations of cancer-related genes.

15. A method of observing the behavior of cells derived from a sample of cancer cells as claimed in claim 2, further comprising the steps of:

f. inoculating cells from one of the prime culture, the reference culture or a subculture of the prime culture or of the reference culture into a plurality of segregated sites; and

g. treating the plurality of sites with at least one treating means, followed by assessment of sensitivity of cells in the site to the treating means.

16. A method of observing the behavior of cells derived from a sample of cancer cells as claimed in claim 15, wherein one or more of the steps are at least partially automated.

17. A method of observing the behavior of cells derived from a sample of cancer cells as claimed in claim 15, further comprising the step of phenotypically or genotypically analyzing the cells in one or more sites for drug resistance.

18. A method of observing the behavior of cells derived from a sample of cancer cells as claimed in claim 15, further comprising the steps of:

h. collecting a specimen of a patient's non-malignant cells;

i. separating the non-malignant cells into cohesive multicellular particulates;

j. growing a tissue culture monolayer from the multicellular particulates of non-malignant cells to form a control culture;

k. inoculating the control culture in a plurality of non-segregated sites;

l. treating the plurality of segregated sites of the control culture with the same treating means as the segregated sites of the prime culture or a subculture thereof, followed by assessment of the sensitivity of the segregated cells of the control culture in the treating means; and

m. comparing the sensitivity of the segregated cells of the prime culture or a subculture thereof with the sensitivity of the segregated cells of the control culture to the treating means.

19. A method of observing the behavior of cells derived from a sample of cancer cells as claimed in claim 18, wherein the assessment of steps g and l are calculations of the percentage or fraction of cells sensitive to the treatment and further comprising the step of:

n. creating a therapeutic index of a ratio of one of the percentage of or the fraction of sensitive cells or insensitive cells in the segregated cells of the control culture to one of the percentage of or the fraction of sensitive cells or insensitive cells in the segregated cells of the prime culture or subculture thereof.

20. A method of observing the behavior of cells derived from a sample of cancer cells as claimed in claim 19, further comprising the step of programming a computer to automatically perform calculations to create said therapeutic index.

21. A method of observing the behavior of cells derived from a sample of cancer

cells as claimed in claim 20, wherein the segregated sites are in a readable plate having a plurality of culture wells and a scanner is used to automatically scan the segregated sites to determine the percentage or fraction of cells sensitive to the treatment and an interface is provided between the scanner and the computer allowing automated input of scanner data into the computer for calculation of the therapeutic index.

22. A method of observing the behavior of cells derived from a sample of cancer cells as claimed in claim 19, wherein the non-malignant cells are epithelial cells.

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ABSTRACT:

A comprehensive and integrated system for monitoring (identifying, tracking and
analyzing) an individual patient's malignancy through the duration of a malignancy
as to a specific patient is provided. The method of the present invention allows for
initial identification of a malignancy, identification of malignancy-specific
cellular or secretal markers, identification of cellular or secreted markers
indicative of complications, study of the invasiveness and aggressiveness of the
malignancy, study of the growth rate of the malignancy, study of the effect of
therapies on the malignancy as compared to control cells of the same patient
(chemosensitivity versus toxicity) and the identification of a therapeutic index
(i.e., the ratio of chemosensitivity:toxicity), study of tumor morphology and study
of histological, cytochemical and immunocytochemical markers.

RELATED APPLICATION

[0001] This is a Continuation-In-Part of U.S. application Ser. No. 08/679,056, filed
Jul. 12, 1996, now U.S. Pat. No. 5,728,541, granted Mar. 17, 1998; U.S. application
Ser. No. 09/095,993, filed Jun. 11, 1998; and U.S. application Ser. No. 09/039,957,
filed Mar. 16, 1998.

BACKGROUND OF THE INVENTION

[0002] 1. Field of the Invention

[0003] A system is provided for in vitro tracking of cancerous tissue over the course of the malignancy. The system provides a method for identifying the malignancy and for determining a patient's prognosis. Further, the system provides for assessing a malignancy's invasiveness, aggressiveness, growth rate, production of extracellular markers, possible side effects and for determining the efficacy on the malignancy of a given therapeutic regimen. The system also allows for generation of a therapeutic index, which serves as an indicator of a given therapy's effectiveness against the malignancy as compared to its undesirable side effects, such as lethality to a patient's normal cells.

Introduction

[0004] Tracking a malignancy in a patient according to prior art methods is an inaccurate process which involves identification of the malignancy through techniques including biopsy and subsequent histological, biochemical, and immunochemical techniques and regularly monitoring the malignancy's progression by invasive (i.e., biopsy) or noninvasive (i.e., x-ray, nuclear imaging, Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET)) methods. These methods are often expensive, inconvenient, painful and usually involve hospital visits and safety risks. It is, therefore, desirable to reduce a patient's exposure to such methods. Furthermore, identification of a malignancy as a known variety of malignancy is often helpful in determining a suitable therapeutic approach and expected prognosis. However, even individually identifiable malignancies differ from patient-to-patient in their growth characteristics and in their responsiveness to treatment.

[0005] Determination of the growth rate, invasiveness and aggressiveness of a given malignancy is critical to prognosis and to the choice of therapies. A patient with a poor prognosis might be given a therapeutic regimen which might be more effective than another regimen but more risky to the patient. A patient with a better prognosis might be given a therapeutic regimen which is less aggressive and less risky to the patient, but which might not be as effective as often as a more dangerous therapy. Similarly, if a malignancy produces factors or creates conditions which cause a dangerous side effect, such as a thrombogenesis, the patient can be treated, preferably prophylactically, for the condition.

[0006] Current methodologies for determining growth rate, invasiveness, aggressiveness or which track the progression of a malignancy include biopsy and short-term culture, which can include drawing of blood or other bodily fluids, or semi- or non-invasive techniques such as x-ray and nuclear imaging. At any given time, a patient could be subject to multiple procedures, depending upon when the information is needed by the physician. Each procedure requires the presence of the patient and usually creates risk or pain. These procedures also can increase the stress level of the patient, which often is an exacerbating factor in cancer and associated prognoses. It is therefore, desirable to reduce the frequency of such procedures.

[0007] Identification of an effective therapeutic regimen is critically important to a patient. Often, once the malignancy is identified, a therapy is chosen based upon prior research on that type of malignancy and is not tailored to the sensitivities of the malignancy of a given patient. Often secondary therapies are needed because a first choice was ineffective. Valuable treatment time can be lost and a patient's life can be threatened.

[0008] All active agents including chemotherapeutic active agents are subjected to rigorous testing as to efficacy and safety prior to approval for medical use in the United States. Methods of assessing efficacy have included elaborate investigations of large populations in double blind studies as to a given treatment method and/or active agent, with concomitant statistical interpretation of the resulting data, but these conclusions are inevitably generalized as to patient populations taken as a whole. In many pharmaceutical disciplines and particularly in the area of chemotherapy, however, the results of individual patient therapy may not comport with generalized data--to the detriment of the individual patient. The need has been long recognized for a method of assessing the therapeutic potential of active agents, including but not limited to chemotherapeutic agents, for their efficacy as to a given individual patient, prior to the treatment of that patient. This need also applies to assessing the therapeutic potential as to radiation therapies, combined radiation/drug therapies and cellular immunotherapies.

[0009] Prior art assays already exist which expose malignant tissue of various types to a plurality of active agents, for the purpose of assessing the best choice for therapeutic administration. For example, in Kruczynski, A., et al., "Evidence of a direct relationship between the increase in the in vitro passage number of human non-small-cell-lung cancer primocultures and their chemosensitivity," Anticancer Research, vol. 13, no. 2, pp. 507-513 (1993), chemosensitivity of non-small-cell-lung cancers was investigated in in vivo grafts, in in vitro primocultures and in commercially available long-term cancer cell lines. The increase in chemosensitivity was documented and correlated with morphological changes in the cells in question. Sometimes animal model malignant cells and/or established cell cultures are tested with prospective therapy agents, see for example Arnold, J.T., "Evaluation of chemopreventive agents in different mechanistic classes using a rat tracheal epithelial cell culture transformation assay," Cancer Res., vol. 55, no. 3, pp. 537-543 (1995).

[0010] In vitro prior art techniques present the further shortcoming that assayed cells do not necessarily express the cellular markers they would express in vivo. This is regrettable because the determination of expression of certain secreted or cellular markers, secreted factors or tumor antigens or lack thereof can be useful for both identification and therapeutic purposes. For instance, members of the fibrinolytic system such as urokinase-type plasminogen activator (u-PA) and plasminogen activator inhibitors type 1 (PAI-1) are up-regulated in malignant brain tumors. See, e.g., Jasti S. Rao, et al., "The Fibrinolytic System in Human Brain Tumors: Association with Pathophysiological Conditions of Malignant Brain Tumors," Advances in Neuro-Oncology II, Kornblith PL, Walker MD (eds) Futura (1997). Other secreted factors such as .alpha.-fetoprotein, carcinoembryonic antigen and transforming growth factors .alpha. and .beta. have been found to be indicative of various cancers and/or cancer progression (see also, Singhal et al., "Elevated Plasma Osteopontin in Metastatic Breast Cancer Associated with Increased Tumor Burden and Decreased Survival," Clinical Cancer Research, vol. 3, 605-611, (April 1997); Kohno et al., "Comparative Studies of CAM 123-6 and Carcinoembryonic Antigen for the Serological Detection of Pulmonary Adenocarcinoma," Cancer Detection and Prevention, 21(2): 124-128 (1997)). These examples are but a few of the many factors that may be used to identify diseased cells.

[0011] Cellular markers also include metastatic markers, indicative of metastatic potential, i.e., invasiveness and aggressiveness, which is relevant to the progression of a given malignancy and to a patient's prognosis. First, markers indicating the invasiveness of a given malignancy indicate the ability of the malignancy to infiltrate and to destroy adjacent tissue. As an example, for epithelial malignancies, invasiveness markers are indicative of the ability of the malignancy to infiltrate beneath the epithelial basement membrane. Invasiveness markers can include the presence of proteolytic enzymes or angiogenic factors. A second category of metastatic marker indicates growth conditions of the malignancy. For instance, a malignancy could require for instance a prostate-specific factor for growth. Invasiveness and aggressiveness factors are often present in serum or in tissue culture media.

[0012] Relevant to a patient's prognosis and, incidentally, to the identification of a malignancy is the presence of markers, cellular or secreted, which lead to complications beyond those involved with uncontrolled growth and invasion by a malignancy. For instance, secretion by the malignancy of thrombogenic substances by the malignancy can result in blood clotting, resulting in thrombophlebitis or other thrombotic events such as pulmonary thrombosis. Identification of a thrombotic potential indicates treatment (preferably prophylactically) with thrombolytic substances.

[0013] When a specific patient's cells are used in in vitro assays in typical prior art processes the cells are harvested (biopsied) and trypsinized (connective tissue digested with the enzyme trypsin) to yield a cell suspension purportedly suitable for conversion to the desired tissue culture form. The in vitro tissue culture cell collections which result from these techniques are generally plagued by their inability accurately to imitate the chemosensitivity or therapeutic sensitivity of the original tumor or other cell biopsy. These collections often do not express cellular markers in the same manner that they would in vivo. A need thus remains for a technique of tissue culture preparation which provides cell cultures, allowing identification of a malignancy, accurate tracking of the malignancy's progress in a patient and therapy screening, in which, after simple preparation, the cell cultures

react in a manner equivalent to their in vivo reactivity. The culture method would enable drug or chemotherapeutic agent, radiation therapy and/or cellular immunotherapy screening as to a particular patient for whom such screening is indicated.

[0014] A need also remains for a technique of tissue culture preparation which provides cell cultures for screening for expressed markers or factors where the cultured cells express the markers or factors in a manner indicative of their in vivo expression of the same. A further need also remains for a tissue culture preparation which allows for morphological study of the cells. Lastly, a need remains for a tissue culture system in which progression of an individual malignancy can be studied as indicative of the in vivo progression of the malignancy.

SUMMARY OF THE INVENTION

[0015] A comprehensive and integrated unified system for monitoring (i.e., identifying, tracking and analyzing) an individual patient's malignancy through the duration of a malignancy as to a specific patient is provided. The method of the present invention allows for initial identification of a malignancy, identification of malignancy-specific cellular or secreted markers, identification of cellular or secreted markers indicative of complications, study of the invasiveness and aggressiveness of the malignancy, study of the growth rate of the malignancy, study of the effect of therapies on the malignancy as compared to control cells of the same patient (chemosensitivity versus toxicity) and the identification of a therapeutic index (i.e., the ratio of chemosensitivity:toxicity), study of tumor morphology and study of histological and cytochemical markers.

[0016] The method of the present invention includes the steps of collecting a tissue sample or specimen of a patient's cells and separating the specimen into cohesive multicellular particulates (explants) of the tissue sample, rather than enzymatically digested cell suspensions or preparations. The cells are then grown as a tissue culture monolayer from the multicellular particulates to form a prime culture. A specimen can be taken from a patient at any relevant site, including but not limited to tissue, ascites or effusion fluid. Samples may also be taken from body fluid or exudates, as is appropriate. A tissue culture monolayer, designated as the prime culture, can be grown in any method known in the art for growing such a monolayer, for instance in tissue culture plates or flasks. If the malignant cells originate from solid tissue, however, the tissue must be subdivided into small pieces from which a tissue culture monolayer is then grown out.

[0017] Once a prime culture is established from a patient's malignancy, the prime culture can be maintained without any treatments beside normal feedings and passage techniques, as indicative of the growth of the malignancy absent treatment. However, subcultures of the prime culture are prepared so that the prime culture is preferably left untreated, and the cells of the prime culture are not affected by any testing. However, either the prime culture or a subculture thereof can be propagated as a reference culture. The reference culture is a culture which is treated with therapies reflective of a patient's actual treatments. For instance, if a patient is treated with a chemotherapeutic agent, the reference culture is treated with the same agent in the same concentration. The reference culture can be monitored genotypically or phenotypically to reflect actual progress of the malignancy in the patient. Treatment of the reference culture need not be limited to anticancer therapies, but can reflect all of a patient's treatments. For instance, thrombolytic or anti-thrombogenic treatments can be applied to the reference culture to reflect a patient's treatment. Subcultures of either the prime culture or the reference culture can be used for further analysis. Preferably, since the reference culture is indicative of the current state of a malignancy at a given time, subcultures of the reference culture are analyzed further. At various points in the passage of the control culture and the reference culture, aliquots of cells from those cultures can be stored cryogenically or otherwise.

[0018] The tissue sample technique of the present invention is also useful in assaying expression and/or secretion of various markers, factors or antigens present on or produced by the cultured cells. These assays can be used for diagnostic purposes for monitoring the applicability of certain candidate therapeutic or chemotherapeutic agents or for monitoring the progress of treatment of the cancer with those agents.

[0019] A method for identifying and monitoring progress of a malignancy in an

individual patient is provided including the steps of inoculating cells from either the prime culture, the reference culture or a subculture of the prime culture or of the reference culture into a plurality of segregated sites; treating the plurality of sites with at least one treating means or therapy, followed by assessment of sensitivity of cells in the site to the treating means; collecting a specimen of a patient's non-malignant cells; separating the non-malignant cells into cohesive multicellular particles; growing a tissue culture monolayer from the multicellular particulates of non-malignant cells to form a control culture; inoculating the control culture in a plurality of non-segregated sites; treating the plurality of segregated sites of the control culture with the same treating means as the segregated sites of the prime culture or a subculture thereof, followed by assessment of the sensitivity of the segregated cells of the control culture to the treating means; and comparing the sensitivity of the segregated cells of the prime culture or a subculture thereof with the sensitivity of the segregated cells of the control culture to the treating means. The assessments described above are calculations of the percentage or fraction of cells sensitive, or insensitive, to the treatment and the method further includes the step of creating a therapeutic index of a ratio of one of the percentage of or the fraction of sensitive cells or insensitive cells in the segregated cells of the control culture to one of the percentage of or the fraction of sensitive cells or insensitive cells in the segregated cells of the prime culture or subculture thereof.

[0020] Lastly, a method for treating a patient having a malignancy is provided, including the steps of: analyzing a patient's cells prepared according to the above-described methods for malignancy-associated markers; determining a therapeutic regimen according to the results of the analysis; and treating a patient according to the regimen. The method can further include the step of treating one of either cells cultured as a subculture of the prime culture or cells of the prime culture according to the regimen as representative of the patient's malignancy. Lastly, the method further includes determining a therapeutic index for each treating means as described above.

[0021] When applicable, cultures can be grown in a readable (scannable) plate and to determine percent confluence of the cells or any other parameter which can be determined in such a manner. The scanner can be operably linked with a computer or CPU to automatically input data into the computer or CPU. The computer or CPU can be programmed to automatically calculate a therapeutic index (or other relevant indices) based upon the data provided by the scanner. Alternatively, the data can be entered manually into the programmed computer or CPU to calculate the index.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] FIGS. 1A, 1B and 1C are graphs of the growth rates of three independent cell cultures.

[0023] FIGS. 2A-2F through 5A-5F are graphs depicting the results of short-term and long-term chemotherapy assays. FIGS. 2A-2F and 3A-3F show short-term and long-term assays for a first patient. FIGS. 4A-4F and 5A-5F show short-term and long-term assays for a second patient.

[0024] FIGS. 6 and 7 show two radiation dose versus surviving fraction curves for two glioblastoma cell lines. Cells were irradiated in microtiter plates and assayed four days post-irradiation.

[0025] FIGS. 8A-8C are graphs of survival rates of cell cultures treated with radiation (FIG. 8A) or with radiation and Taxol (FIGS. 8B and 8C).

[0026] FIGS. 9A and 9B are graphs showing data from a series of experiments where target cells from two tumor types were exposed to Activated Natural Killer (ANK) cells.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0027] The present invention is an improved and unified system for monitoring the progression of an individual malignancy and for identifying cellular and secreted markers, markers indicative of certain side effects of the malignancy and for screening multiple candidate therapeutic or chemotherapeutic agents for efficacy and long term effect as to a specific patient. In the method, a tissue sample from the patient is harvested, cultured and separately exposed to a plurality of treatments

and/or therapeutic agents for the purpose of objectively identifying the best treatment for the cultured cells obtained from the patient. The culture techniques of the present invention also result in a monolayer of cells that express cellular markers, secreted factors and tumor antigens in a manner representative of their expression in vivo. Specific method innovations such as tissue sample preparation techniques render this method practically as well as theoretically useful. One particularly important tissue sample preparation technique is the initial preparation of cohesive multicellular particulates of the tissue sample, rather than enzymatically dissociated cell suspensions or preparations, for initial tissue culture monolayer preparation. With respect to the culturing of malignant cells, for example, it is believed (without any intention of being bound by the theory) that by maintaining the malignant cells within a multicellular particulate of the originating tissue, growth of the malignant cells themselves is facilitated versus the overgrowth of fibroblasts or other cells which tends to occur when suspended tumor cells are grown in culture. Practical monolayers of cells may thus be formed to enable meaningful screening of a plurality of treatments and/or agents as well as meaningful identification of cellular markers. In the drug assays, growth of cells is monitored to ascertain the time to initiate the assay and to determine the growth rate of the cultured cells; sequence and timing of drug addition is also monitored and optimized. By subjecting uniform samples of cells to a wide variety of active agents (and concentrations thereof), the most efficacious agent can be determined. For assays concerning cancer treatment, a two-stage evaluation is contemplated in which both acute cytotoxic and longer term inhibitory effects of a given anti-cancer agent are investigated.

[0028] With regard to the identification of expressed cellular markers, secreted factors or tumor antigens, with the initial culturing of the multicellular particulates it is believed (without any intention of being bound by the theory) that because the cells are grown under conditions closer to those found in vivo, the cells express their cellular markers, secreted factors and tumor antigens in a manner more closely resembling their expression in vivo. By assaying the culture media obtained from growing a monolayer according to the inventive method or by histochemically and/or immunohistochemically assaying the cells grown under such conditions, a more accurate profile of the cellular markers or factors is obtained.

[0029] Thus, a comprehensive and integrated system for identifying, tracking and analyzing an individual patient's malignancy through the duration of the malignancy and thereafter is provided. The method of the present invention allows for initial identification of a malignancy, identification of malignancy-specific cellular or secreted markers, identification of cellular or secreted markers indicative of complications, study of the invasiveness and aggressiveness of the malignancy, study of the growth rate of the malignancy, study of the effect of therapies on the malignancy as compared to control cells of the same patient (chemosensitivity and/or radiosensitivity versus toxicity) and the identification of a therapeutic index (i.e., the ratio of chemosensitivity:toxicity), study of tumor morphology and study of histological, cytochemical and immunocytochemical markers.

[0030] The method of the present invention includes the steps of collecting a tissue sample or specimen of a patient's cells and separating the specimen into cohesive multicellular particulates (explants) of the tissue sample, rather than enzymatically digested cell suspensions or preparations. The cells are then grown as a tissue culture monolayer from the multicellular particulates to form a prime culture. A specimen can be taken from a patient at any relevant site, including but not limited to tissue, ascites or effusion fluid. Samples may also be taken from body fluid or exudates, as is appropriate. A tissue culture monolayer can be grown in any method known in the art for growing such a monolayer, for instance in tissue culture plates or flasks.

[0031] Once a prime culture is established from a patient's malignancy, the prime culture can be maintained without any treatments beside normal feedings and passage techniques, as indicative of the growth of the malignancy absent treatment with a therapeutic regimen. Subcultures of the prime culture are prepared so that the cells of the prime culture are not affected by any subsequent testing or treatments. Although prime culture is preferably left untreated, either the prime culture or a subculture thereof can be propagated as a reference culture. The reference culture is a culture which is treated with therapies reflective of a patient's actual treatment regimen. For instance, if a patient is treated with a chemotherapeutic agent, the reference culture is treated with the same agent in the same concentration. The reference culture can be monitored genotypically or

phenotypically to reflect actual progress of the malignancy in the patient. Treatment of the reference culture need not be limited to anticancer therapies, but can reflect all of a patient's treatments. For instance, thrombolytic or anti-thrombogenic treatments, can be applied to the reference culture to reflect a patient's treatment. Subcultures of either the prime culture or the reference culture can be used for further analysis. Preferably, since the reference culture is indicative of the current state in a patient of a malignancy, subcultures of the reference culture are analyzed. At various points in the passage of the control culture and the reference culture, aliquots of cells from those cultures can be stored cryogenically, or otherwise.

[0032] An important further aspect of the present invention is to provide a system for screening specific tissue samples from individual patients for expressed cellular markers, secreted factors or antigens, including tumor antigens, characteristic of the tissue sample. A tissue sample from a patient is harvested and grown in a monolayer culture as described above. Culture medium in which the cultures or subcultures thereof are assayed for the presence or absence of certain factors, such as secreted tumor antigens such as PAI-1, u-PA, cancer associated serum antigen (CASA) or carcinoembryonic antigen (CEA). These factors may be detected through use of standard assays such as radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA), although other assays known to those skilled in the art may be used to detect and/or to quantify the soluble factors. The cell cultures grown in this manner may also be assayed histochemically and or immunohistochemically for identification or quantification of cellular or membrane-bound markers. Examples of such markers include, without limitation, CEA, tissue polypeptide specific antigen, (TPS) and mucin antigens, such as CA 15-3, CA 549, CA 27.29 and MCA. By screening tissue samples in this manner, for production of such factors, markers or antigens, the cultured cells may be further identified, aiding the physician in treatment strategies and as a prognosis indicator. Furthermore, by combining the use of the culture technique with assaying for such markers, factors and antigens, a treatment strategy for a disease state may be optimized and treatment progression may be monitored.

[0033] One important aspect of analyzing tissue culture medium is that complications of a malignancy can be predicted. For instance, one common complication is thrombogenesis. A propensity towards blood clot formation can be detected in tissue culture medium by identifying thrombogenic or procoagulant factors such as, without limitation, the Lewis Y antigen (Ley), HLA-DR and other tumor procoagulants, such as cancer procoagulant (CP) and tissue factor (TF). By identifying production of thrombogenic factors, a physician can prescribe drug and/or exercise regimens, as appropriate, to prevent life and/or limb-threatening clotting.

[0034] Cells and/or tissue culture media from any of the prime culture, the reference culture or subcultures thereof can be analyzed for tumor aggressiveness and invasiveness markers. Presence of these markers or absence thereof is highly relevant to a patient's prognosis. Furthermore, the effect of a given therapy on any of these markers can be analyzed. For instance, a tumor may produce angiogenic factors, such as, without limitation, vascular endothelial growth factor (VEGF), which would lead a doctor to give a patient a less favorable prognosis. Other markers can include, without limitation, factors which allow cancer cells to affix to organs other than those from which the cancer cells derive, for instance, beta 3 integrin, which participates in the ability of melanoma cells to adhere to blood vessel walls. However, the effectiveness of therapies can be assessed if the presence of the angiogenic marker is analyzed in segregated sites according to the method of the present invention. A physician can suppress a malignancy by preventing expression of factors or markers which cause the malignancy's aggressiveness or invasiveness.

[0035] An important application of the present invention is the screening of chemotherapeutic agents and other anti-neoplastic therapies in tissue culture preparations of malignant cells from the patients from whom malignant samples are biopsied. Related anti-cancer therapies which also can be screened using the inventive system include radiation therapy and agents which enhance the cytotoxicity of radiation, as well as immunotherapeutic anti-cancer agents. Screening processes for treatments or therapeutic agents for nonmalignant syndromes are also embraced within this invention and include without limitation agents which combat hyper-proliferative syndromes, such as psoriasis, or wound healing agents. Nor is the present efficacy assay limited only to the screening of active agents which speed up (healing) or slow down (anti-cancer, anti-hyper-proliferative) cell growth

because agents intended to enhance or to subdue intracellular biochemical functions may be tested in the present tissue culture system also. For example, the formation or blocking of enzymes, neurotransmitters and other biochemicals may be screened with the present assay methods prior to treatment of the patient.

[0036] When a patient is to be treated for the presence of tumor, in the preferred embodiment of the present invention a tumor biopsy of >100 mg of non-necrotic, non-contaminated tissue is harvested from the patient by any suitable biopsy or surgical procedure known in the art. Biopsy sample preparation generally proceeds as follows under a Laminar Flow Hood which should be turned on at least 20 minutes before use. Reagent grade ethanol is used to wipe down the surface of the hood prior to beginning the sample preparation. The tumor is then removed, under sterile conditions, from the shipping container and is minced with sterile scissors. If the specimen arrives already minced, the individual tumor pieces should be divided into four groups. Using sterile forceps, each undivided tissue quarter is then placed in 3 ml sterile growth medium (Standard F-10 medium containing 17% calf serum and a standard amount of Penicillin and Streptomycin) and systematically minced by using two sterile scalpels in a scissor-like motion, or mechanically equivalent manual or automated opposing incisor blades. This cross-cutting motion is important because the technique creates smooth cut edges on the resulting tumor multicellular particulates. Preferably but not necessarily, the tumor particulates each measure 1 mm.sup.3. After each tumor quarter has been minced, the particles are plated in culture flasks using sterile pasteur pipettes (9 explants per T-25 or 20 particulates per T-75 flask). Each flask is then labeled with the patient's code, the date of explanation and any other distinguishing data. The explants should be evenly distributed across the bottom surface of the flask, with initial inverted incubation in a 37.degree. C. incubator for 5-10 minutes, followed by addition of about 5-10 ml sterile growth medium and further incubation in the normal, non-inverted position. Flasks are placed in a 35.degree. C., non-CO.sub.2 incubator. Flasks should be checked daily for growth and contamination. Over a period of a few weeks, with weekly removal and replacement of 5 ml of growth medium, the explants will foster growth of cells into a monolayer. With respect to the culturing of malignant cells, it is believed (without any intention of being bound by the theory) that by maintaining the malignant cells within a multicellular particulate of the originating tissue, growth of the malignant cells themselves is facilitated versus the overgrowth of fibroblasts (or other unwanted normal cells) which tends to occur when suspended tumor cells are grown in culture.

[0037] The use of the above procedure to form a cell monolayer culture maximizes the growth of malignant cells from the tissue sample, and thus optimizes ensuing tissue culture assay of chemotherapeutic action of various agents to be tested. Enhanced growth of actual malignant cells is only one aspect of the present invention; however, another important feature is the growth rate monitoring system used to oversee growth of the monolayer once formed. Once a primary culture and its derived secondary monolayer tissue culture has been initiated, the growth of the cells is monitored to ascertain the time to initiate the chemotherapy assay and to determine the growth rate of the cultured cells.

[0038] Monitoring of the growth of cells is conducted by counting the cells in the monolayer on a periodic basis, without killing or staining the cells and without removing any cells from the culture flask. The counting may be done visually or by automated methods, either with or without the use of estimating techniques known in the art (counting in a representative area of a grid multiplied by number of grid areas, for example). Data from periodic counting is then used to determine growth rates which may or may not be considered parallel to growth rates of the same cells in vivo in the patient. If growth rate cycles can be documented, for example, then dosing of certain active agents can be customized for the patient. The same growth rate can be used to evaluate radiation treatment periodicity, as well. It should be noted that with the growth rate determinations conducted while the monolayers grow in their flasks, the present method requires no hemocytometry, flow cytometry or use of microscope slides and staining, with all their concomitant labor and cost.

[0039] Protocols for monolayer growth rate generally use a phase-contrast inverted microscope to examine culture flasks incubated in a 37.degree. C. (5% CO.sub.2) incubator. When the flask is placed under the phase-contrast inverted microscope, ten fields (areas on a grid inherent to the flask) are examined using the 10.times. objective, with the proviso that the ten fields should be non-contiguous, or significantly removed from one another, so that the ten fields are a representative sampling of the whole flask. Percentage cell occupancy for each field examined is

noted, and averaging of these percentages then provides an estimate of overall percent confluency in the cell culture. When patient samples have been divided between two or among three or more flasks, an average cell count for the total patient sample should be calculated. The calculated average percent confluency should be entered into a process log to enable compilation of data--and plotting of growth curves--over time. Monolayer cultures may be photographed to document cell morphology and culture growth patterns. The applicable formula is: 1 Percent confluency = estimate of the area occupied by cells total area in an observed field.

[0040] As an example, therefore, if the estimate of area occupied by the cells is 30% and the total area of the field is 100%, percent confluency is 30/100, or 30.

[0041] Adaptation of the above protocol for non-tumor cells is straightforward and generally constitutes an equivalent procedure.

[0042] Active agent and/or radiation therapy screening using the cultured cells proceeds with subcultures of the prime culture or, preferably, of the reference culture. The screening can be carried out in an incubation flask, but generally proceeds using plates such as microtiter plates. In a chemotherapy/radiotherapy assay, it is desirable to grow a control culture of a patient's cells in a culture parallel to the reference or prime culture. The control culture can be grown from skin cells, as an easy source of non-malignant cells, from the same organ from which the malignant cells are derived, or from other sources, so long as the cells are typical of non-malignant cells of the patient.

[0043] The performance of the chemosensitivity/radiosensitivity assay used for screening purposes depends on the ability to deliver a reproducible cell number to each row in a plate and/or a series of plates, as well as the ability to achieve an even distribution of cells throughout a given well. The following procedure assures that cells are reproducibly transferred from flask to microtiter plates, and cells are evenly distributed across the surface of each well.

[0044] The first step in preparing the microtiter plates is, of course, preparing and monitoring the monolayer as described above. The following protocol is exemplary and susceptible of variation as will be apparent to one skilled in the art. Cells are removed from the culture flask and a cell pellet is prepared by centrifugation. The cell pellet derived from the monolayer is then suspended in 5 ml of the growth medium and mixed in a conical tube with a vortex for 6 to 10 seconds. The tube is then rocked back and forth 10 times. A 36 .mu.l droplet from the center of the conical tube is pipetted onto one well of a 96 well plate. A fresh pipette is then used to pipette a 36 .mu.l aliquot of trypan blue solution, which is added to the same well, and the two droplets are mixed with repeated pipette aspiration. The resulting admixture is then divided between two hemocytometer chambers for examination using a standard light microscope. Cells are counted in two out of four hemocytometer quadrants, under 10.times. magnification. Only those cells which have not taken up the trypan blue dye are counted. This process is repeated for the second counting chamber. An average cell count per chamber is thus determined. Using means known in the art, the quadrant count values are checked, logged, multiplied by 10.sup.4 to give cells/ml, and the total amount of fluid (growth medium) necessary to suspend remaining cell aliquots is calculated accordingly.

[0045] After the desired concentration of cells in medium has been determined, additional cell aliquots from the monolayer are suspended in growth medium via vortex and rocking and loaded into a Terasaki dispenser known in the art. Aliquots of the prepared cell suspension are delivered into the microtiter plates using Terasaki dispenser techniques known in the art. A plurality of plates may be prepared from a single cell suspension as needed. Plates are then wrapped in sterile wet cotton gauze and incubated in an incubator box by means known in the art.

[0046] After the microtiter plates have been prepared, exposure of the cells therein to active agent and/or radiation is conducted according to the following exemplary protocol. During this portion of the inventive assay, the appropriate amount of specific active agent is transferred into the microtiter plates prepared as described above. A general protocol, which may be adapted, follows. Each microtiter plate is unwrapped from its wet cotton gauze sponge and microscopically examined for cell adhesion. Control solution is dispensed into delineated rows of wells within the grid in the microtiter plate, and appropriate aliquots of active agent to be tested are added to the remaining wells in the remaining rows. Ordinarily, sequentially increasing concentrations of the active agent or higher doses of

radiation being tested are administered into progressively higher numbered rows in the plate. The plates are then rewrapped in their gauze and incubated in an incubator box at 37.degree. C. under 5% CO_2. After a predefined exposure time, the plates are unwrapped, blotted with sterile gauze to remove the agent, washed with Hank's Balance Salt Solution, flooded with growth medium, and replaced in the incubator in an incubator box for a predefined time period, after which the plates may be fixed and stained for evaluation.

[0047] Fixing and staining may be conducted according to a number of suitable procedures; the following is representative. After removal of the plates from the incubator box, culture medium is poured off and the plates are flooded with Hank's Balance Salt Solution. After repeated flooding (with agitation each time) the plates are then flooded with reagent grade ethanol for 2-5 minutes. The ethanol is then poured off. Staining is accomplished with approximately 5 ml of Giemsa Stain per plate, although volume is not critical and flooding is the goal. Giemsa stain should be left in place 5 min. .+-.30 seconds as timing influences staining intensity. The Giemsa stain is then poured off and the plates are dipped three times in cold tap water in a beaker. The plates are then inverted, shaken vigorously, and air dried overnight (with plate lids off) on a rack on a laboratory bench. Cells per well are then counted manually or by automated and/or computerized means, to derive data regarding chemosensitivity of cells at various concentrations of exposure. One particularly useful computer operating environment for counting cells is the commercially available OPTIMATE compiler, which is designed to permit an optical counting function well suited to computerized cell counting procedures and subsequent calculations.

[0048] The above procedures do not change appreciably when cell growth promoters are assayed rather than cell arresting agents such as chemotherapeutic or radiotherapeutic agents. The present assay allows cell death or cell growth to be monitored with equal ease. In any case, optimization of use of the present system will involve the comparative testing of a variety of candidate active agents for selection of the best candidate for patient treatment based upon the in vitro results. One particularly advantageous embodiment of the above described invention comprises a two-stage assay for cytotoxicity followed by evaluation of longer-term inhibitory effect. Chemotherapeutic agents may thus be evaluated separately for both their direct chemotherapeutic effect as well as for their longer duration efficacy.

[0049] As discussed in brief, above, in parallel with growth of the prime or reference culture, a control culture can be grown. The control culture is a culture of normal cells taken from the same patient from whom the prime culture is collected. The control culture can derive from an epithelial cell sample or can be collected from the same organ as the prime culture so long as the control culture contains no malignant cells. More than one control culture can be maintained. For instance, cultures of both normal skin cells and normal cells of an organ from which the malignancy is derived can be maintained. The value of maintaining a control culture is many fold. Primarily, the control culture serves as a negative control (or positive control, depending upon the marker to be analyzed) in the various analyses to be carried out on the prime culture, the reference culture or subcultures thereof.

[0050] A second value of the control culture is an indicator of toxicity, the toxicity or undesirable effects of a given therapy upon normal cells. For instance, in the segregated analysis of chemotherapeutic agents described above, concomitant analysis of the same agents on segregated sites of the control culture would yield an indication of cytotoxicity of the agent with regard to malignant cells versus the toxicity of the agent to control cells. A therapeutic index can be calculated based on the ratio of cytotoxicity to malignant cells to toxicity. Cytotoxicity and toxicity can be quantified as a percentage or fraction of cells killed by a given therapy, or as a percentage or fraction of cells surviving a given therapy. A therapeutic index is a ratio of these percentages or fractions and is reflective of the desirability of a given treatment in a patient. An optimal treatment would be maximally cytotoxic (or even cytostatic) to the malignant cells and minimally toxic to a patient's normal cells.

[0051] Other indices may be generated, depending upon the desired effect of a therapy. For instance, if a desired therapy is designed to up-regulate a malignancy-specific antigen to promote destruction of the malignancy by a patient's immune system, an index could be generated to discern a treatment which reflects maximal up-regulation of the antigen in the malignant cells and minimal or negative

up-regulation in a patient's normal cells. A similar index can be calculated based upon down-regulation of a desired marker (i.e., an angiogenic factor) which can be assayed as either a secreted or a cellular marker and reflects maximal down-regulation of the marker with minimal toxicity or other undesirable effects on the control culture.

[0052] Often the diseased cells express a cellular marker that is indicative of a certain disease state or lack thereof. However, one aspect of the culture techniques of the present invention is that the cultured diseased cells do not necessarily have to be the cells expressing the factor to be assayed. One question that inevitably arises when considering whether a serum marker is indicative of a particular cancer cell is, which cells produce the marker, the cell or the tissue in which the cancer cells grow? See e.g. Singhal et al., p 610. By co-culturing the cancerous tissue within a multicellular particulate of its originating tissue, the cells (both the diseased cells or the surrounding cells) are better able to retain their production of characteristic markers.

[0053] Identification of one or more active agents or chemotherapeutic agents is peripheral to the present invention, which is intended for the efficacy screening of any or all of them as to a given patient. Literally any active agent may be screened according to the present invention; listing exemplary active agents is thus omitted here.

[0054] One important focus of the present invention thus includes the simplicity of the present system--cohesive multicellular particulates of the patient tissue to be tested are used to form cell monolayers; growth of those monolayers is monitored for accurate prediction of correlating growth of the same cells in vivo; and differing concentrations of a number of active agents may be tested for the purpose of determining not only the most appropriate agent but the most appropriate concentration of that agent for actual patient exposure (according to the calculated cell growth rates). It is also important to note, in the context of the invention, that the present system allows in vitro tests to be conducted in suspensions of tissue culture monolayers grown in nutrient medium under fast conditions (a matter of weeks), rather than with single cell progeny produced by dilution cloning over long periods of time. In some cases, the present invention is a two stage assay for both cytotoxicity and the longer-term growth inhibitory.

[0055] It is additionally possible to increase the value of the assay with the use of staining compositions and protocols designed to characterize the malignant cells thus grown. In other words, the tissue preparation and cell culturing technique itself offers a first assurance that the cells grown out of the tumor are really the malignant tumor cells and not fibroblasts or other nonmalignant cells of no diagnostic value. As a separate confirmation, the present staining compositions and protocols offer a second, independent assurance that the cells subject to diagnostic or prognostic assay are in fact malignant cells in culture. One important characterization has to do with the nature of the malignant cells as epithelial, which is in turn an indicator of the carcinoma type of malignancy. Other characterizations of malignant cells are intended to fall within the scope of the present invention as well, although the characterization of the cells as epithelial or not is of primary importance.

[0056] The technique is practiced as follows. The same cell culturing and well distribution process is used as in the cytotoxicity assay described above, but rather than exposing the cells to chemotherapeutic or other agents, the cells are instead fixed and stained. With the stain or stain cocktail described below, the epithelial cells are identified by their intermediate filaments and/or specific membrane antigens by means of a monoclonal antibody immunoperoxidase technique. The fixative used can be any fixative which does not alter the cellular molecular markers of interest. The fixed, stained cells are then counted. If the specimen is positive for epithelial cells, the process is complete. If the specimen is negative for epithelial cells, an independent fixing and staining process is subsequently completed, with fresh cells from identical wells, using Vimentin as a stain to confirm the non-epithelial nature of the cells.

[0057] The importance of having a stain or stain cocktail (i.e., antibody cocktail), as well as an overall protocol, for identifying epithelial cells in biopsies of malignant tumors is as follows. In the basic cytotoxicity assay, the tissue culture technique is designed to grow out the cells of the tumor of origin and in fact consistently does so. Despite such reliable predictability, however, the fact that

the cells of the tumor of origin did in fact grow out, and not fibroblasts or other cells, must be confirmed with independent proof before the cells can be used with complete assurance in the appropriate patient assay(s). The present technology provides a means to obtain this confirmation, which in turn furthers the interests of good laboratory and medical practice.

[0058] As a general consideration, the staining compounds or compositions of interest for use in the present technology are those which bind with cellular molecular markers unique either to epithelial or to non-epithelial cells. A further aspect of the invention therefore inheres in the following two aspects: the improvement of the cytotoxicity assay by adding the epithelial staining protocol with any known epithelial stain; and the further improvement wherein specially designed stain cocktails maximize the likelihood that the presence of any known intermediate filament or specific membrane antigen, characteristic of epithelial cells, will be identified if present.

[0059] Many carcinomas are positive for any one of the intermediate filaments or specific membrane antigens characteristic of epithelial cells; virtually all if not all carcinomas are positive for one of a number of such intermediate filaments or specific membrane antigens. For example, "epithelial membrane antigen" (EMA) glycoproteins are known in the art and can be bound with various antiepithelial membrane antigen antibodies including monoclonal antibodies. Cytokeratin is another important epithelial cell marker and binding reagents including monoclonal antibodies are available which are specific to cytokeratin. While antisera can be raised in vivo against markers such as EMA glycoproteins and cytokeratin, as a practical matter commercially available polyclonal or monoclonal antibodies are used in the following protocols, with monoclonal antibodies being preferred.

[0060] Binding of the epithelial marker is revealed with associated staining procedures and reactions which give a visual indication that the marker binding took place. Those skilled in the art already appreciate various techniques already available--in the general field of "immunocytochemistry" --to reveal antibody-antigen reactions. One known way to accomplish this visualization when antibody binding reagents are used is with the "labeled streptavidin procedure". In this procedure, after the specimen is exposed to antibodies specific to the target antigen, a secondary "link" antibody is added. The secondary biotinylated "link" antibody consists of anti-mouse and anti-rabbit antibodies which bind universally to most primary monoclonal or polyclonal antibodies. The "link" will also connect to the tertiary reagent (peroxidase-labeled streptavidin) through chemical bonding between the biotin on the secondary reagent and the streptavidin on the streptavidin/peroxidase conjugate. Staining is completed by incubating the specimen and primary, secondary and tertiary agents in the presence of a chromogen, so that the peroxidase and the chromogen form a visible precipitate. Alternatively, a fluorescein-based detection system can be used to visualize the primary antibody, or a third alternative known in the art as the digoxigenin-conjugated detection system may be used.

[0061] Of the various epithelial markers, three have received the most widespread attention in the literature: EMA glycoproteins, cytokeratin, and carcinoembryonic antigen. In the context of this invention, the first two are the most important because literally any epithelial cell will have at least either one EMA glycoprotein on the surface thereof or a cytokeratin intermediate filament present. Therefore, the present invention resides not only in binding and staining for an epithelial marker on the surfaces of the specimen cells, but in simultaneously assaying for either or both of EMA glycoprotein(s) and cytokeratin. The cocktails of the present invention therefore contain binding reagents for both EMA glycoproteins and cytokeratin and, importantly, are selected to include the most generally applicable binding reagents in combination so that the cocktail has the broadest binding scope possible. The cocktails identified in Examples 1 and 2, for example, represent a combination of two general binding reagents (containing a total of three monoclonal antibodies) for cytokeratin, admixed with a general binding reagent for EMA glycoprotein. The dual benefit of this admixture of general binding agents is that the incidence of false negatives for epithelial cells is minimized, and the visible staining reactions are generally stronger when the combined binding reagents are used in lieu of a single binding reagent.

[0062] Although the binding reagents and other reagents identified in the Examples are the preferred reagents for use in the context of the invention, the invention is intended to encompass epithelial-specific binding and staining reagents generally.

These include, without limitation: Boehringer-Mannheim AE1 anti-cytokeratin antibody; Boehringer-Mannheim AE3 anti-cytokeratin antibody; Boehringer-Mannheim AE1/AE3 anti-cytokeratin antibody (AE1 and AE3 in admixture); Becton-Dickinson CAM 5.2 antibody, DAKO EMA antibody, Biomeda's Anti-Cytokeratin Cocktail CK22, Biomeda's Anti-Cytokeratin Cocktail CK23, Biomeda's Anti-Pan-Cytokeratin CK56, Biomeda's polyclonal goat or rabbit anti-cytokeratin antisera, ScyTek Laboratories' anti-EMA antigen antibody clone E29, and many others. Those skilled in the art and in possession of the guidance provided herein can readily determine alternative, equivalent binding and staining reagents and cocktails, to accomplish the disclosed result. These binding agents and cocktails may be used in combination with any known visualization system, such as the streptavidin, fluorescein- and digoxigenin-conjugated systems identified above.

[0063] As a control, Vimentin antibody is used as a binding alternative either in conjunction with binding and staining of the test cells, or subsequently thereto. In the context of this invention, Vimentin can be considered a binding reagent which is specific to non-epithelial cells of mesenchymal origin.

[0064] In a further aspect of the present invention, immunological markers may be monitored in applications requiring up- or down- regulation of such markers (i.e., Major histocompatibility complex molecules). This aspect of the present invention can be especially useful in transplantation applications where, for instance, through chemical or biological means rejection of transplanted cells is sought to be avoided by down-regulation of the various transplantation antigens present on the cells to be transplanted. The present invention would be especially useful in monitoring such immunoregulation.

[0065] Lastly, cell morphology can be assayed by culturing cells of, i.e., the prime culture or the reference culture, removing the cells from the surface upon which they grow, centrifuging cells into a loose pellet and growing the cell pellet over a defined time period. By growing cells in this manner, it is possible to view the cohesive morphology of cells in a cluster resembling a tumor.

EXAMPLE 1

Radiation Therapy

[0066] Separate 50 mg samples from residual tissue from specimens of three human glioblastomas and one human ovarian carcinoma were minced in medium with sterile scissors to a particle size of roughly 1 mm.sup.3 and with a particle size distribution between about 0.25 and about 1.5 mm.sup.3. The medium was Standard F-10 medium containing 17% calf serum and a standard amount of Penicillin and Streptomycin. Each 50 mg sample was minced and was divided into four groups of particulates and each of 16 groups was charged to a separate labeled culture flask containing the above-described medium. Visual confirmation was made that the particulates were evenly distributed along the bottom of each flask and the flasks were placed in a 35..degree. C., non-CO.sub.2 incubator. Flasks were checked daily for growth and contamination. Over a period of a few weeks, with weekly removal and replacement of 5 ml of growth medium, the particulates grew into monolayers.

[0067] Enough cells were then removed from the monolayers grown in the flasks for centrifugation into standard size cell pellets for each of the 16 flasks. Each cell pellet was then suspended in 5 ml of the above-described medium and was mixed in a conical tube with a vortex for 6 to 10 seconds, followed by manual rocking back and forth 10 times. A 36 ml droplet from the center of each tube was then pipetted into one well of a 96-well microtiter plate together with an equal amount of trypan blue, plus stirring. The resulting admixture was then divided between two hemocytometer quadrants for examination using a standard light microscope. Cells were counted in two out of four hemocytometer quadrants, under 10.times. magnification--only those cells which had not taken up the trypan blue dye were counted. This process was repeated for the second counting chamber. An average cell count per chamber was calculated and by means known in the art the optimum concentration of cells in the medium was determined.

[0068] Accommodating the above calculations, additional cell aliquots from the 16 monolayers were separately suspended in growth medium via vortex and rocking and were loaded into a Terasaki dispenser adapted to a 60-well plate. Aliquots of the prepared cell suspension were delivered into the microtiter plates using Terasaki dispenser techniques known in the art. Cells were plated into 60-well microtiter

plates at a concentration of 100 cells per well.

[0069] Twenty-four (24) hours later, the cells were irradiated using a Siemens
Stabilipan X-ray machine at 250 kVp, 15 mA with a dose rate of 75 rad/minute. For
each radiation dose from 1Gy to 6Gy, cell number per well was monitored as a
function of time through five days post-irradiation.

[0070] Cell number relative to controls was determined and survival curves were fit
to the data. The rate of decrease in survival as a function of time was proportional
to dose. A differential radiation response among the four cell lines was observed.

EXAMPLE 2

Immuno Therapy

[0071] Separate 50 mg samples from residual tissue from specimens of a human brain
tumor, renal carcinoma, and breast carcinoma were minced in medium with sterile
scissors to a particle size of roughly 1 mm.sup.3 and with a particle size
distribution between about 0.25 and about 1.5 mm.sup.3. The medium was Standard F-10
medium containing 17% calf serum and a standard amount of Penicillin and
Streptomycin. Each 50 mg sample was minced and was divided into four groups of
particulates and each of 12 groups was charged to a separate labeled culture flask
containing the above-described medium. Visual confirmation was made that the
particulates were evenly distributed along the bottom of each flask and the flasks
were placed in a 35.degree. C., non-CO.sub.2 incubator. Flasks were checked daily
for growth and contamination. Over a period of a few weeks, with weekly removal and
replacement of 5 ml of growth medium, the particulates grew into monolayers.

[0072] Enough cells were then removed from the monolayers grown in the flasks for
centrifugation into standard size cell pellets for each of the twelve flasks. Each
cell pellet was then suspended in 5 ml of the above-described medium and was mixed
in a conical tube with a vortex for 6 to 10 seconds, followed by manual rocking back
and forth 10 times. A 36 ml droplet from the center of each tube was then pipetted
into one well a 96-well microtiter plate together with an equal amount of trypan
blue, plus stirring. The resulting admixture was then divided between two
hemocytometer quadrants for examination using a standard light microscope. Cells
were counted in two out of four hemocytometer quadrants, under 10.times.
magnification--only those cells which had not taken up the trypan blue dye were
counted. This process was repeated for the second counting chamber. An average cell
count per chamber was calculated and by means known in the art the optimum
concentration of cells in the medium was determined.

[0073] Accommodating the above calculations, additional cell aliquots from the 12
monolayers were separately suspended in growth medium via vortex and rocking and
were loaded into a Terasaki dispenser adapted to a 60-well plate. Aliquots of the
prepared cell suspension were delivered into the microtiter plates using Terasaki
dispenser techniques known in the art. Cells were plated into 60-well microtiter
plates at a concentration of 100 cells per well.

[0074] Twenty-four (24) hours post-plating, Activated Natural Killer (ANK) cells
were delivered into a row of six wells by means of a micropipette. In each
microtiter plate three rows of six wells each served as controls. The effector (ANK
cells):target cell (tumor cells) ratio varied from 2.5:1 to 20:1. The ANK cells were
exposed to the target cells for four hours. Subsequently, the wells were washed with
Hanks Balanced Salt Solution and the number of ANK cells remaining in the wells was
observed with a phase contrast microscope. This process was repeated until no ANK
cells remained in the wells (usually 3 washes). Following removal of the ANK cells,
the tumor cells were incubated in the wells for another 24 hours.

[0075] Cell number relative to control was determined. For the three tumor types
increasing the effector: target cell ratio from 2.5:1 to 20:1 resulted in an
increase in the number of tumor cells killed by the ANK cells.

EXAMPLE 3

Gene Therapy/Antisense Oligonucleotides

[0076] A 50 mg sample from a residual human mesothelioma was minced in medium with
sterile scissors to a particle size of roughly 1 mm.sup.3 and with a particle size

distribution between about 0.25 and about 1.5 mm.sup.3. The medium was Standard F-10 medium containing 17% calf serum and a standard amount of Penicillin and Streptomycin. The 50 mg sample was minced and was divided into four groups of particulates and each of four groups was charged to a separate labeled culture flask containing the above-described medium. Visual confirmation was made that the particulates were evenly distributed along the bottom of each flask and the flasks were placed in a 35.degree. C., non-CO.sub.2 incubator. Flasks were checked daily for growth and contamination. Over a period of a few weeks, with weekly removal and replacement of 5 ml of growth medium, the particulates grew into monolayers.

[0077] Enough cells were then removed from the monolayers grown in the flasks for centrifugation into standard size cell pellets for each of the four flasks. Each cell pellet was then suspended in 5 ml of the above-described medium and was mixed in a conical tube with a vortex for 6 to 10 seconds, followed by manual rocking back and forth 10 times. A 36 ml droplet from the center of each tube was then pipetted into one well of a 96-well microtiter plate together with an equal amount of trypan blue, plus stirring. The resulting admixture was then divided between two hemocytometer quadrants for examination using a standard light microscope. Cells were counted in two out of four hemocytometer quadrants, under 10.times. magnification--only those cells which had not taken up the trypan blue dye were counted. This process was repeated for the second counting chamber. An average cell count per chamber was calculated and by means known in the art the optimum concentration of cells in the medium was determined.

[0078] Accommodating the above calculations, additional cell aliquots from the 4 monolayers were separately suspended in growth medium via vortex and rocking and were loaded into a Terasaki dispenser adapted to a 60-well plate. Aliquots of the prepared cell suspension were delivered into the microtiter plates using Terasaki dispenser techniques known in the art. Cells were plated into 60-well microtiter plates at a concentration of 100 cells per well.

[0079] Twenty-four (24) hours post-plating, antisense oligonucleotide for the urokinase-type plasminogen activator receptor (uPAR) was delivered to wells in the microtiter plate. Proteolysis of plasminogen to plasmin by urokinase-type plasminogen activator has been implicated in the processes of tumor cell proliferation and invasion. The concentrations of the uPAR antisense oligonucleotide were 1, 10 and 100 micromolar. uPAR sense and missense oligonucleotides at the concentrations of 1, 10 and 100 micromolar served as controls. The tumor cells were exposed to the oligonucleotides for 24 hours and then the agents were removed. The cells were allowed to incubate for another 72 hours so that inhibition of cell proliferation could be observed.

[0080] Cell number relative to control was then determined. Antisense oligonucleotides to uPAR suppressed the proliferative activity of the tumor cells in a concentration dependent manner.

EXAMPLE 4

Combination Chemotherapy

[0081] Separate 50 mg samples from residual tissue from specimens from four human ovarian tumors were minced in medium with sterile scissors to a particle size of roughly 1 mm.sup.3 and with a particle size distribution between about 0.25 and about 1.5 mm.sup.3. The medium was Standard F-10 medium containing 17% calf serum and a standard amount of Penicillin and Streptomycin. Each 50 mg sample was minced and was divided into 4 groups of particulates and each of 16 groups was charged to a separate labeled culture flask containing the above-described medium. Visual confirmation was made that the particulates were evenly distributed along the bottom of each flask and the flasks were placed in a 35.degree. C., non-CO.sub.2 incubator. Flasks were checked daily for growth and contamination. Over a period of a few weeks, with weekly removal and replacement of 5 ml of growth medium, the particulates grew into monolayers.

[0082] Enough cells were then removed from the monolayers grown in the flasks for centrifugation into standard size cell pellets for each of the 16 flasks. Each cell pellet was then suspended in 5 ml of the above-described medium and was mixed in a conical tube with a vortex for 6 to 10 seconds, followed by manual rocking back and forth 10 times. A 36 ml droplet from the center of each tube was then pipetted into one well of a 96-well microtiter plate together with an equal amount of trypan blue,

plus stirring. The resulting admixture was then divided between two hemocytometer quadrants for examination using a standard light microscope. Cells were counted in two out of four hemocytometer quadrants, under 10.times. magnification--only those cells which had not taken up the trypan blue dye were counted. This process was repeated for the second counting chamber. An average cell count per chamber was calculated and by means known in the art the optimum concentration of cells in the medium was determined.

[0083] Accommodating the above calculations, additional cell aliquots from the 16 monolayers were separately suspended in growth medium via vortex and rocking and were loaded into a Terasaki dispenser adapted to a 60-well plate. Aliquots of the prepared cell suspension were delivered into the microtiter plates using Terasaki dispenser techniques known in the art. Cells were plated into 60-well microtiter plates at a concentration of 100 cells per well.

[0084] Twenty-four (24) hours post-plating, the chemotherapeutic agent Taxol was applied to the wells in the microtiter plates. The first three treatment rows in the plates (Rows 2, 3, and 4) were designed to have escalating Taxol doses (1.0, 5.0, and 25 .mu.M) with a fixed carboplatin dose (200 .mu.M). The last three treatment rows in the plates (Rows 6, 7, and 9) were designed to have a fixed Taxol dose (5 .mu.M) with an escalating carboplatin dose (50, 200, and 1000 .mu.M). Rows 5 and 9 served as a control. The Taxol exposure time was two hours. Twenty-four hours later, the cells in the wells were exposed to carboplatin for two hours. The tumor cells in the wells were then incubated for another 48 hours.

[0085] Cell number relative to control was determined. For the cells from the four tumor specimens a dose response relationship was observed for both the escalating Taxol/fixed carboplatin and fixed Taxol/escalating carboplatin treatment schema.

EXAMPLE 5

Hormonal Therapy

[0086] Separate 50 mg samples from residual tissue from specimens from four human breast tumors were minced in medium with sterile scissors to a particle size of roughly 1 mm.sup.3 and with a particle size distribution between about 0.25 and about 1.5 mm.sup.3. The medium was Standard F-10 medium containing 17% calf serum and a standard amount of Penicillin and Streptomycin. Each 50 mg sample was minced and was divided into four groups of particulates and each of 16 groups was charged to a separate labeled culture flask containing the above-described medium. Visual confirmation was made that the particulates were evenly distributed along the bottom of each flask and the flasks were placed in a 35.degree. C., non-CO.sub.2 incubator. Flasks were checked daily for growth and contamination. Over a period of a few weeks, with weekly removal and replacement of 5 ml of growth medium, the particulates grew into monolayers.

[0087] Enough cells were then removed from the monolayers grown in the flasks for centrifugation into standard size cell pellets for each of the 16 flasks. Each cell pellet was then suspended in 5 ml of the above-described medium and was mixed in a conical tube with a vortex for 6 to 10 seconds, followed by manual rocking back and forth 10 times. A 36 ml droplet from the center of each tube was then pipetted into one well of a 96-well microtiter plate together with an equal amount of trypan blue, plus stirring. The resulting admixture was then divided between two hemocytometer quadrants for examination using a standard light microscope. Cells were counted in two out of four hemocytometer quadrants, under 10.times. magnification--only those cells which had not taken up the trypan blue dye were counted. This process was repeated for the second counting chamber. An average cell count per chamber was calculated and by means known in the art the optimum concentration of cells in the medium was determined.

[0088] Accommodating the above calculations, additional cell aliquots from the 16 monolayers were separately suspended in growth medium via vortex and rocking and were loaded into a Terasaki dispenser adapted to a 60-well plate. Aliquots of the prepared cell suspension were delivered into the microtiter plates using Terasaki dispenser techniques known in the art. Cells were plated into 60-well microtiter plates at a concentration of 100 cells per well.

[0089] Twenty-four (24) hours post-plating, the antiestrogenic compound Tamoxifen was delivered to wells in the microtiter plates. A stock solution of Tamoxifen was

initially prepared by dissolving 1.5 mg of Tamoxifen powder in 1 ml of absolute ethanol and then adding 9 ml of growth medium. This stock solution was then used to make Tamoxifen solutions in the concentration range of 10 nM to 20 .mu.M. Six doses of Tamoxifen were used for cells from each of the four breast tumor specimens. An ethanol solution at a concentration equivalent to that at the highest Tamoxifen concentration served as a control. The tumor cells were exposed to Tamoxifen for 24 hours and then the agent was removed. The cells were allowed to incubate for another 72 hours so that inhibition of cell proliferation could be observed.

[0090] Cell number relative to control was then determined. There was no effect observed when the ethanol-only control wells were compared to the growth medium-only control wells. The cells of two of the four breast specimens tested showed an inhibition of cell proliferation by Tamoxifen exposure. These responses occurred in the mid to high Tamoxifen concentration ranges.

EXAMPLE 6

Differentiating Agent Therapy ("Biological Response Modification")

[0091] Separate 50 mg samples from residual tissue from specimens from four human breast tumors were minced in medium with sterile scissors to a particle size of roughly 1 mm.sup.3 and with a particle size distribution between about 0.25 and about 1.5 mm.sup.3. The medium was Standard F-10 medium containing 17% calf serum and a standard amount of Penicillin and Streptomycin. Each 50 mg sample was minced and was divided into four groups of particulates and each of 16 groups was charged to a separate labeled culture flask containing the above-described medium. Visual confirmation was made that the particulates were evenly distributed along the bottom of each flask and the flasks were placed in a 35.degree. C., non-CO.sub.2 incubator. Flasks were checked daily for growth and contamination. Over a period of a few weeks, with weekly removal and replacement of 5 ml of growth medium, the particulates grew into monolayers.

[0092] Enough cells were then removed from the monolayers grown in the flasks for centrifugation into standard size cell pellets for each of the 16 flasks. Each cell pellet was then suspended in 5 ml of the above-described medium and was mixed in a conical tube with a vortex for 6 to 10 seconds, followed by manual rocking back and forth 10 times. A 36 ml droplet from the center of each tube was then pipetted into one well of a 96-well microtiter plate together with an equal amount of trypan blue, plus stirring. The resulting admixture was then divided between two hemocytometer quadrants for examination using a standard light microscope. Cells were counted in two out of four hemocytometer quadrants, under 10.times. magnification--only those cells which had not taken up the trypan blue dye were counted. This process was repeated for the second counting chamber. An average cell count per chamber was calculated and by means known in the art the optimum concentration of cells in the medium was determined.

[0093] Accommodating the above calculations, additional cell aliquots from the 16 monolayers were separately suspended in growth medium via vortex and rocking and were loaded into a Terasaki dispenser adapted to a 60-well plate. Aliquots of the prepared cell suspension were delivered into the microtiter plates using Terasaki dispenser techniques known in the art. Cells were plated into 60-well microtiter plates at a concentration of 100 cells per well.

[0094] Twenty-four (24) hours post-plating the differentiating agent retinoic acid was delivered to wells in the microtiter plates. A stock solution of retinoic acid was initially prepared by dissolving retinoic acid powder in 1 ml of dimethyl sulfoxide (DMSO) and then adding 9 ml of growth medium. This stock solution was then used to make retinoic acid solutions in the concentration range of 0.1 to 1.0 mM. Six doses of retinoic acid were used for cells from each of the four breast tumor specimens. A DMSO solution at a concentration equivalent to that at the highest retinoic acid concentration served as a control. The tumor cells were exposed to retinoic acid for 24 hours and then the agent was removed. The cells were allowed to incubate for another 72 hours so that inhibition of cell proliferation could be observed.

[0095] Cell number relative to control was then determined. There was no effect observed when the DMSO-only control wells were compared to the growth medium-only control wells. The cells of three of the four breast specimens tested showed an inhibition of cell proliferation by retinoic acid exposure. These responses occurred

in the mid to high retinoic acid concentration ranges.

EXAMPLE 7

Combined Modality Therapy Drug/Radiation

[0096] Separate 50 mg samples from residual tissue from specimens from two human brain tumors and two human ovarian tumors were minced in medium with sterile scissors to a particle size of roughly 1 mm.sup.3 and with a particle size distribution between about 0.25 and about 1.5 mm.sup.3. The medium was Standard F-10 medium containing 17% calf serum and a standard amount of Penicillin and Streptomycin. Each 50 mg sample was minced and was divided into four groups of particulates and each of 16 groups was charged to a separate labeled culture flask containing the above-described medium. Visual confirmation was made that the particulates were evenly distributed along the bottom of each flask and the flasks were placed in a 35.degree. C., non-CO.sub.2 incubator. Flasks were checked daily for growth and contamination. Over a period of a few weeks, with weekly removal and replacement of 5 ml of growth medium, the particulates grew into monolayers.

[0097] Enough cells were then removed from the monolayers grown in the flasks for centrifugation into standard size cell pellets for each of the 16 flasks. Each cell pellet was then suspended in 5 ml of the above-described medium and was mixed in a conical tube with a vortex for 6 to 10 seconds, followed by manual rocking back and forth 10 times. A 36 ml droplet from the center of each tube was then pipetted into one well of a 96-well microtiter plate together with an equal amount of trypan blue, plus stirring. The resulting admixture was then divided between two hemocytometer quadrants for examination using a standard light microscope. Cells were counted in two out of four hemocytometer quadrants, under 10.times. magnification--only those cells which had not taken up the trypan blue dye were counted. This process was repeated for the second counting chamber. An average cell count per chamber was calculated and by means known in the art the optimum concentration of cells in the medium was determined.

[0098] Accommodating the above calculations, additional cell aliquots from the 16 monolayers were separately suspended in growth medium via vortex and rocking and were loaded into a Terasaki dispenser adapted to a 60-well plate. Aliquots of the prepared cell suspension were delivered into the microtiter plates using Terasaki dispenser techniques known in the art. Cells were plated into 60-well microtiter plates at a concentration of 100 cells per well.

[0099] Twenty-four (24) hours post-plating, cells in the microtiter plate wells were exposed to the chemotherapeutic agent Taxol. One set of plates was designed to have escalating Taxol doses with (0.5-25.0 .mu.M) with a fixed radiation dose (2Gy). A second set of plates was designed to have a fixed Taxol dose (5 .mu.M) with an escalating radiation dose (1Gy-6Gy). The cells in the plates were irradiated using a Siemans Stabilipan X-ray machine operating at 250 kVp, 15 mA with a dose rate of 75 rad/minute.

[0100] For each of the two treatment schema, cell number per well was monitored as a function of time through 5 days post-treatment. Cell number relative to controls was determined and survival curves were fit. A differential response among the cells from the four tumor specimens was observed. Both additive and synergistic cell killing was noted.

EXAMPLE 8

Initiation of a Prime Culture

[0101] A tumor biopsy of approximately 100 mg of non-necrotic, non-contaminated tissue was harvested from the patient by surgical biopsy and transferred to the laboratory in a standard shipping container. Biopsy sample preparation proceeded as follows. Reagent grade ethanol was used to wipe down the surface of a Laminar flow hood. The tumor was then removed, under sterile conditions, from its shipping container, and cut into quarters with a sterile scalpel. Using sterile forceps, each undivided tissue quarter was then placed in 3 ml sterile growth medium (Standard F-10 medium containing 17% calf serum and a standard amount of Penicillin and Streptomycin) and was systematically minced by using two sterile scalpels in a scissor-like motion. The tumor particulates each measured about 1 mm.sup.3. After each tumor quarter was minced, the particles were plated in culture flasks using

• sterile pasteur pipettes (9 explants per T-25 or 20 particulates per T-75 flask). Each flask was then labeled with the patient's code, the date of explanation and any other distinguishing data. The explants were evenly distributed across the bottom surface of the flask, with initial inverted incubation in a 37.degree. C. incubator for 5-10 minutes, followed by addition of about 5-10 ml sterile growth medium and further incubation in the normal, non-inverted position. Flasks were placed in a 35.degree. C., non-CO.sub.2 incubator. Flasks were checked daily for growth and contamination. Over a period of a few weeks, with weekly removal and replacement of 5 ml of growth medium, the explants grew out into a monolayer.

EXAMPLE 9

Unified Tracking System

[0102] a. Growth Rate

[0103] Following initiation of prime cell culture of a tumor specimen, the growth rate of the cells was determined until the chemosensitivity assay was performed. During this time period the growth was monitored by observing the percent of confluency of the cells in a flask. These data provide information valuable as a correlation to possible growth of the tumor in the patient as well as for the interpretation of the results of the chemosensitivity assay.

[0104] Three examples of growth rate data are shown in FIGS. 1A-1C. The percent of confluency of the cultured cells is plotted as a function of time after the initial seeding of the tissue specimen.

[0105] Slow Growth Rate (FIG. 1A): 25% confluent after 19 days

[0106] Moderate Growth Rate (FIG. 1B): 60% confluent after 21 days

[0107] Fast Growth Rate (FIG. 1C): 90% confluent after 11 days

[0108] b. Immunohistochemical Staining for Cell Characterization, etc.

[0109] Many tumor specimens will contain a mixture of cancer and normal cells. Although in many cases tumor cells will readily grow in tissue culture, while the normal cells will not, it is important to be able to distinguish the two cell types. Using immunoperoxidase techniques to stain cells for various intermediate filaments, the differences between normal (fibroblast-like) cells and cells from epithelial tumors were characterized. These techniques can also be used to identify other tumor cell characteristics which may have prognostic value.

[0110] An initial attempt at cultured cell characterization has been to use known epithelial tumor cell lines and a known fibroblast cell line. The epithelial tumor cell lines all have stained positively for a mixture ("cocktail") of epithelial intermediate filament antibodies, (not every line, however, has stained positively for the three antibodies within the mixture [AE1/AE3; Cam 5.2; EMA]). Some of the epithelial tumor cells in culture also stained mildly positive for an antibody against an intermediate filament characteristic of fibroblasts (vimentin). When staining for fibroblast intermediate filament (vimentin) in cell culture, all fibroblast cells were positive. Some focal staining by epithelial tumor cells for vimentin was also present.

1

	epithelial cocktail	vimentin
epithelial tumor cells	++	+
fibroblasts	-	++

[0111] Testing of intermediate filaments with antibodies for epithelial cells and vimentin appears to be a method of distinguishing certain characteristics of tumor and normal cells.

[0112] c. Response to Chemotherapy

[0113] The tissue culture chemosensitivity assay has been refined to make it more sensitive for the detection of damage produced by a variety of chemotherapeutic agents. The initial alteration was to allow a 24-hour time period between plating of cells in microtiter wells and the exposure to drugs. This time interval permits cells to be in an active state of proliferation, where they are more sensitive to cell cycle active agents. The second change was to initiate a long-term assay (growth inhibition assay) over a period of about 72 hours. The short-term assay is conducted 24-72 hours after the therapeutic agent is added. The longer time between drug exposure and assay allows for the detection of cell damage which occurs over a protracted period and requires several cell division cycles before it becomes apparent. "CI" is a measure of the relative survival rates of a given cell culture. It is calculated by according to the formula: 2 CI = (1 - No. of cells in treated wells) No. of cells in control wells

[0114] The data for a short-term assay and a long-term assay performed on two sets of patient cultured cells are presented in FIGS. 2A-2F through 5A-5F. The long-term assay (FIGS. 3A-3F and 5A-5F) may both accentuate a positive result obtained from the short-term assay (FIGS. 2A-2F and 4A-4F) and reveal an effect not observed during the short-term assay. The long-term assay is now incorporated into the tissue culture chemosensitivity on a routine basis.

[0115] d. Response to Radiation Therapy

[0116] The use of the microtiter well assay to analyze the direct effect of radiation therapy on tumor cells in culture has resulted in a rapid evaluation method for the determination of inherent cellular radiation response. As an example, two radiation dose-response curves generated from the microtiter well assay are presented in FIGS. 6 and 7. The cells from the tumor specimen in FIG. 6 are more resistant than those of the specimen in FIG. 7. The more resistant tumor has been previously irradiated.

[0117] The microtiter well assay is ideally suited for examination of the interaction of chemotherapeutic agents and radiation. Issues such as the differential sensitivity of drug/radiation combinations and the timing of drug/radiation combinations may be directly addressed with this system. An illustration of chemotherapeutic agent enhancement of radiation response is presented in FIGS. 8A-8C.

2

    FIG. 8A: Radiation-only at 2 Gy and 4 Gy
    FIG.
8B: Taxol 8.5 ng/ml + 2 Gy and 4 Gy
    FIG. 8C: Taxol 42.5 ng/ml + 2
Gy and 4 Gy

[0118] e. Response to Cellular Immunotherapy

[0119] Activated lymphocytes are being used as a treatment for some types of cancer. These Activated Natural Killers (ANK) cells have been shown to mediate highly efficient cell killing for some tumor types. The microtiter well assay can be utilized to make a rapid assessment of ANK-induced tumor target cell killing. An illustration of two such interactions is presented in FIGS. 9A and 9B.

[0120] In FIGS. 9A and 9B, the target cells were from a melanoma and a renal carcinoma, respectively. The target cells were exposed to the ANK cells for 4 hours and then the assay was performed. The effector:target cell ratio varied from 1:20 to

1:2.5. The data show increasing cell killing as a function of increasing effector: target ratio.

[0121] f. Use of Tissue Culture Medium for Determination of Factors with Possible Prognostic/Biological Significance

[0122] A number of substances secreted by tumor cells such as Tumor Associated Antigens and Plasminogen Activators and Inhibitors are believed to regulate a variety of processes involved in the progression of malignant disease. Many of these factors are produced by tumor cells growing in tissue culture and are secreted into the growth medium. The measurement of these factors in the medium from cell cultures of tumor specimens may prove to be of predictive value in the assessment of the biological behavior of individual cancers.

[0123] Preliminary work in this area has been on the detection of plasminogen activator inhibitor in the growth medium of glioblastoma cell lines. Plasminogen activator inhibitor expression has been shown to be increased in malignant brain tumors in patients. Medium from glioblastoma cell lines showed an increase in plasminogen activator inhibitor when compared to the medium alone.

[0124] Any or all of the steps of the unified assays and culturing techniques of the present invention may be automated. Indices can be automatically calculated by a computer which is programmed appropriately. Data can be input into the computer either manually or automatically, into a spreadsheet or database program, or the like. The spreadsheet or database program can be programed to reduce the data to the indices described above, or to any other relevant form, i.e., graphical or figurative representations of the data.

[0125] In one example, the cells to be assayed are grown on microtiter plates and assayed for their sensitivity to a chemotherapeutic agent according to the above-described protocols. The microtiter plates are read on an optical scanner and data from the scanner is automatically exported to a computer for calculation of a therapeutic index. Other types of scanners may be utilized depending upon the assay. For instance, a scanner for reading RIA data would be provided if the assay is an RIA assay.

[0126] Although the present invention has been described with respect to specific materials and methods above, the invention is only to be considered limited insofar as is set forth in the accompanying claims.

CLAIMS:

I claim:

1. A method for identifying and monitoring progress of an individual patient having, a malignancy comprising the steps of: a. collecting a specimen of a patient's cells; b. separating the specimen into cohesive multicellular particulates; c. growing a tissue culture monolayer from the multicellular particulates to form a prime culture; and d. monitoring said tissue culture monolayer over a period of time.

2. A method for identifying and monitoring progress of a malignancy in an individual patient as claimed in claim 1 further comprising the step of maintaining the prime culture.

3. A method for identifying and monitoring progress of a malignancy in an individual patient as claimed in claim 1 further comprising the steps of preparing a reference culture from the prime culture and treating the reference culture with one or more treatments as given to the patient.

4. A method for identifying and monitoring progress of a malignancy in an individual patient as claimed in claim 3 further comprising the steps of preparing a subculture of one of the prime culture and the reference culture.

5. A method for identifying and monitoring progress of a malignancy in an individual patient as claimed in claim 4 further comprising the step of assaying for a malignancy-specific one of the prime culture, the reference culture, the subculture and tissue culture medium used to grow one of the prime culture, the reference culture or the subculture.

6. A method for identifying and monitoring progress of a malignancy in an individual patient as claimed in claim 5 wherein the marker indicates one of aggressiveness and invasiveness of the malignancy.

7. A method for identifying and monitoring progress of a malignancy in an individual patient as claimed in claim 5 wherein the marker is indicative of complications associated with the malignancy.

8. A method for identifying and monitoring progress of a malignancy in an individual patient as claimed in claim 7 wherein the marker is indicative of a thrombogenic potential.

9. A method for identifying and monitoring progress of a malignancy in an individual patient as claimed in claim 5 wherein the marker is identified by one of cytochemistry or immunohistochemistry.

10. A method for identifying and monitoring progress of a malignancy in an individual patient as claimed in claim 9 wherein the marker is selected from the group consisting of an estrogen receptor, a progesterone receptor, an oncogene, a product of an oncogene, a marker for multi-drug resistance and a marker for phenotypic characterization.

11. A method for identifying and monitoring progress of a malignancy of an individual patient as claimed in claim 5 wherein one or more of the steps are at least partially automated.

12. A method for identifying and monitoring progress of a malignancy in an individual patient as claimed in claim 5 wherein the marker is characterized by a molecular biological technique.

13. A method for identifying and monitoring progress of a malignancy in an individual patient as claimed in claim 12 wherein the molecular biological technique characterizes one of tumor cell heterogeneity or specific mutations of cancer-related genes

14. A method for identifying and monitoring progress of a malignancy in an individual patient as claimed in claim 2 further comprising the steps of: d. inoculating cells from one of the prime culture, the reference culture or a subculture of the prime culture or of the reference culture into a plurality of segregated sites; and e. treating the plurality of sites with at least one treating means, followed by assessment of sensitivity of cells in the site to the treating means.

15. A method for identifying and monitoring progress of a malignancy in an individual patient as claimed in claim 14 wherein one or more of the steps are at least partially automated.

16. A method for identifying and monitoring progress of a malignancy in an individual patient as claimed in claim 14 further comprising the step of phenotypically or genotypically analyzing the cells in one or more sites for drug resistance.

17. A method for identifying and monitoring progress of a malignancy in an individual patient as claimed in claim 14 further comprising the steps of: f. collecting a specimen of a patient's non-malignant cells; g. separating the non-malignant cells into cohesive multicellular particulates; h. growing a tissue culture monolayer from the multicellular particulates of non-malignant cells to form a control culture; i. inoculating the control culture in a plurality of non-segregated sites; j. treating the plurality of segregated sites of the control culture with the same treating means as the segregated sites of the prime culture or a subculture thereof, followed by assessment of the sensitivity of the segregated cells of the control culture to the treating means; and k. comparing the sensitivity of the segregated cells of the prime culture or a subculture thereof with the sensitivity of the segregated cells of the control culture to the treating means.

18. A method for identifying and monitoring progress of a malignancy in an individual patient as claimed in claim 17 wherein the assessment of steps e and j are calculations of the percentage or fraction of cells sensitive to the treatment and further comprising the step of: l. creating a therapeutic index of a ratio of

one of the percentage of or the fraction of sensitive cells or insensitive cells in the segregated cells of the control culture to one of the percentage of or the fraction of sensitive cells or insensitive cells in the segregated cells of the prime culture or subculture thereof.

19. A method for identifying and monitoring progress of a malignancy in an individual patient as claimed in claim 18 further comprising the step of programming a computer to automatically perform calculations to create said therapeutic index.

20. A method for identifying and monitoring progress of a malignancy in an individual patient as claimed in claim 19 wherein the segregated sites are in a readable plate having a plurality of culture wells and a scanner is used to automatically scan the segregated sites to determine the percentage or fraction of cells sensitive to the treatment and an interface is provided between the scanner and the computer allowing automated input of scanner data into the computer for calculation of the therapeutic index.

21. A method for identifying and monitoring progress of a malignancy in an individual patient as claimed in claim 18 wherein the non-malignant cells are epithelial cells.

22. A method for treating a patient having a malignancy comprising the steps of: a. analyzing a patient's cells prepared according to the method of claim 1 for malignancy-associated markers; b. determining a therapeutic regimen according to the results of the analysis; and c. treating a patient according to the regimen.

23. A method for treating a patient having a malignancy as claimed in claim 22 further comprising the step of treating one of cells cultured as a subculture of the prime culture and cells of the prime culture according to the method of claim 14.

24. A method for treating a patient having a malignancy as claimed in claim 22 wherein the analyzing step further includes the steps of: i. inoculating cells from one of the prime culture, the reference culture or a subculture of the prime culture or the reference culture into a plurality of segregated sites; ii. treating the plurality of sites with at least one treating means, followed by assessment of sensitivity of cells in the site to the treating means; and iii. determining a therapeutic index for each treating means according to the method of claim 18.

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ABSTRACT:

An improved system for screening a multiple of candidate therapeutic or chemotherapeutic agents for efficacy as to a specific patient, in which a tissue sample from the patient is harvested, cultured and separately exposed to a plurality of treatments and/or therapeutic agents for the purpose of objectively identifying the best treatment or agent for the particular patient. Specific method innovations such as tissue sample preparation techniques render this method practically as well as theoretically useful. The identity of the malignant cells in culture is advantageously confirmed using binding reagents/staining systems specific for epithelial cells, since carcinomas are ubiquitously epithelial in nature. Cells of interest and thus confirmed as epithelial/carcinomal may then be assayed for sensitive to an infinite variety of malignancy treating agents including chemotherapeutic agents, radiation, immunotherapy, and so on.

FIELD OF THE INVENTION

[0001] The invention relates to screening and testing of active agents, including chemotherapeutic agents, to predict potential efficacy in individual patients in whom treatment with such agents is indicated, and staining compositions and protocols to confirm the identity of the cultured malignant cells of interest.

INTRODUCTION

[0002] All active agents including chemotherapeutic active agents are subjected to rigorous testing as to efficacy and safety prior to approval for medical use in the U.S. Methods of assessing efficacy have included elaborate investigations of large populations in double blind studies as to a given treatment method and/or active agent, with concomitant statistical interpretation of the resulting data, but these conclusions are inevitably generalized as to patient populations taken as a whole.

• In many pharmaceutical disciplines and particularly in the area of chemotherapy, however, the results of individual patient therapy may not comport with generalized data--to the detriment of the individual patient. The need has been long recognized for a method of assessing the therapeutic potential of active agents, including but not limited to chemotherapeutic agents, for their efficacy as to a given individual patient, prior to the treatment of that patient.

[0003] Prior art assays already exist which expose malignant tissue of various types to a plurality of active agents, for the purpose of assessing the best choice for therapeutic administration. For example, in Kruczynski, A., et al., "Evidence of a direct relationship between the increase in the in vitro passage number of human non-small-cell-lung cancer primocultures and their chemosensitivity," Anticancer Research, vol. 13, no. 2, pp. 507-513 (1993), chemosensitivity of non-small-cell-lung cancers was investigated in in vivo grafts, in in vitro primocultures and in commercially available long-term cancer cell lines. The increase in chemosensitivity was documented and correlated with morphological changes in the cells in question. Sometimes animal model malignant cells and/or established cell cultures are tested with prospective therapy agents, see, for example, Arnold, J. T., "Evaluation of chemopreventive agents in different mechanistic classes using a rat tracheal epithelial cell culture transformation assay," Cancer Res.., vol. 55, no. 3, pp. 537-543 (1995).

[0004] When actual patient cells are used to form in vitro assays focussed on individual patients, in typical prior art processes the cells are harvested (biopsied) and trypsinized (connective tissue digested with the enzyme trypsin) to yield a cell suspension suitable for conversion to the desired tissue culture form. The in vitro tissue culture cell collections which result from these techniques are generally plagued by their inability accurately to imitate the chemosensitivity of the original tumor or other cell biopsy. Standard cloning and tissue culture techniques are moreover excessively complicated and expensive for use in a patient-by-patient assay setting. A need thus remains for a technique of tissue culture preparation which provides cell cultures, for drug screening purposes, in which after simple preparation the cell cultures react in a manner equivalent to their in vivo reactivity, to enable drug or chemotherapeutic agent screening as to a particular patient for whom such screening is indicated.

SUMMARY OF THE INVENTION

[0005] In order to meet this need, the present invention is an improved system for screening a multiple of candidate therapeutic or chemotherapeutic agents for efficacy as to a specific patient and for confirming the identity of the malignant cells being tested, in which a tissue sample from the patient is harvested, cultured and separately exposed to a plurality of treatments and/or therapeutic agents for the purpose of objectively identifying the best treatment for the cultured cells obtained from the patient. Specific method innovations such as tissue sample preparation techniques render this method practically as well as theoretically useful. One particularly important tissue sample preparation technique is the initial preparation of cohesive multicellular particulates of the tissue sample, rather than enzymatically dissociated cell suspensions or preparations, for initial tissue culture monolayer preparation. With respect to the culturing of malignant cells, for example, it is believed (without any intention of being bound by the theory) that by maintaining the malignant cells within a multicellular particulate of the originating tissue, growth of the malignant cells themselves is facilitated versus the overgrowth of fibroblasts or other cells which tends to occur when suspended tumor cells are grown in culture. Practical monolayers of cells may thus be formed to enable meaningful screening of a plurality of treatments and/or agents. Growth of cells is monitored to ascertain the time to initiate the assay and to determine the growth rate of the cultured cells; sequence and timing of drug addition is also monitored and optimized. By subjecting uniform samples of cells to a wide variety of active agents (and concentrations thereof), the most efficacious agent can be determined. For assays concerning cancer treatment, a two-stage evaluation is contemplated in which both acute cytotoxic and longer term inhibitory effect of a given anti-cancer agent are investigated.

[0006] As an important part of the above technique, staining compositions and protocols are used (1) to identify whether the malignant cells grown in culture are epithelial cells and, if not, (2) to confirm that the cells grown in culture are specifically non-epithelial. The overall method, including the method of characterizing the cells grown in culture with these staining compositions and

protocols as well as unique antibody cocktails therefor, forms the core of the subject matter of this continuation-in-part specification.

DETAILED DESCRIPTION OF THE INVENTION

[0007] The present invention is a system for screening a multiple of candidate therapeutic or chemotherapeutic agents for efficacy as to a specific patient, in which a tissue sample from the patient is harvested and separately exposed to a plurality of treatments and/or therapeutic agents for the purpose of objectively identifying the best treatment or agent. Specific method innovations such as tissue sample preparation techniques render this method practically as well as theoretically useful. One particularly important tissue sample preparation technique is the initial preparation of cohesive multicellular particulates (explants) of the tissue sample, rather than enzymatically dissociated cell suspensions or preparations, for initial tissue culture monolayer preparation. Cell growth, and sequence and timing of drug addition, are monitored and optimized.

[0008] As an important part of the above technique, staining compositions and protocols are used (1) to identify whether the malignant cells grown in culture are epithelial cells and, if not, (2) to confirm that the cells grown in culture are specifically non-epithelial. The overall method, including the method of characterizing the cells grown in culture with these staining compositions and protocols as well as unique antibody cocktails therefor, forms the core of the subject matter of this continuation-in-part specification.

[0009] An important application of the present invention is the screening of chemotherapeutic agents and other antineoplastic therapies against tissue culture preparations of malignant cells from the patients from whom malignant samples are biopsied. Related anti-cancer therapies which can be screened using the inventive system are both radiation therapy and agents which enhance the cytotoxicity of radiation, as well as immunotherapeutic anti-cancer agents. Screening processes for treatments or therapeutic agents for nonmalignant syndromes are also embraced within this invention, however, and include without limitation agents which combat hyperproliferative syndromes, such as psoriasis, or wound healing agents. Nor is the present efficacy assay limited only to the screening of active agents which speed up (healing) or slow down (anti-cancer, anti-hyperproliferative) cell growth because agents intended to enhance or to subdue intracellular biochemical functions may be tested in the present tissue culture system also. For example, the formation or blocking of enzymes, neurotransmitters and other biochemicals may be screened with the present assay methods prior to treatment of the patient.

[0010] When the patient is to be treated for the presence of tumor, in the preferred embodiment of the present invention a tumor biopsy of >100 mg of non-necrotic, non-contaminated tissue is harvested from the patient by any suitable biopsy or surgical procedure known in the art. Biopsy sample preparation generally proceeds as follows under a Laminar Flow Hood which should be turned on at least 20 minutes before use. Reagent grade ethanol is used to wipe down the surface of the hood prior to beginning the sample preparation. The tumor is then removed, under sterile conditions, from the shipping container and is minced with sterile scissors. If the specimen arrives already minced, the individual tumor pieces should be divided into four groups. Using sterile forceps, each undivided tissue quarter is then placed in 3 ml sterile growth medium (Standard F-10 medium containing 17% calf serum and a standard amount of Penicillin and Streptomycin) and systematically minced by using two sterile scalpels in a scissor-like motion, or mechanically equivalent manual or automated opposing incisor blades. This cross-cutting motion is important because the technique creates smooth cut edges on the resulting tumor multicellular particulates. Preferably but not necessarily, the tumor particulates each measure 1 mm.sup.3. After each tumor quarter has been minced, the particles are plated in culture flasks using sterile pasteur pipettes (9 explants per T-25 or 20 particulates per T-75 flask). Each flask is then labeled with the patient's code, the date of explanation and any other distinguishing data. The explants should be evenly distributed across the bottom surface of the flask, with initial inverted incubation in a 37.degree. C. incubator for 5-10 minutes, followed by addition of about 5-10 ml sterile growth medium and further incubation in the normal, non-inverted position. Flasks are placed in a 35.degree. C., non-CO.sub.2 incubator. Flasks should be checked daily for growth and contamination. Over a period of a few weeks, with weekly removal and replacement of 5 ml of growth medium, the explants will foster growth of cells into a monolayer. With respect to the culturing of malignant cells, it is believed (without any intention of being bound by the theory)

that by maintaining the malignant cells within a multicellular particulate of the originating tissue, growth of the malignant cells themselves is facilitated versus the overgrowth of fibroblasts (or other unwanted cells) which tends to occur when suspended tumor cells are grown in culture.

[0011] The use of the above procedure to form a cell monolayer culture maximizes the growth of malignant cells (and only malignant cells) from the tissue sample, and thus optimizes ensuing tissue culture assay of chemotherapeutic action of various agents to be tested. Enhanced growth of actual malignant cells is only one aspect of the present invention, however; another important feature is the growth rate monitoring system used to oversee growth of the monolayer once formed. Once a primary culture and its derived secondary monolayer tissue culture has been initiated, the growth of the cells is monitored to ascertain the time to initiate the chemotherapy assay and to determine the growth rate of the cultured cells.

[0012] Monitoring of the growth of cells is conducted by counting the cells in the monolayer on a periodic basis, without killing or staining the cells and without removing any cells from the culture flask. The counting may be done visually or by automated methods, either with or without the use of estimating techniques known in the art (counting in a representative area of a grid multiplied by number of grid areas, for example). Data from periodic counting is then used to determine growth rates which may or may not be considered parallel to growth rates of the same cells in vivo in the patient. If growth rate cycles can be documented, for example, then dosing of certain active agents can be customized for the patient. The same growth rate can be used to evaluate radiation treatment periodicity, as well. It should be noted that with the growth rate determinations conducted while the monolayers grow in their flasks, the present method requires no hemocytometry, flow cytometry or use of microscope slides and staining, with all their concomitant labor and cost.

[0013] Protocols for monolayer growth rate generally use a phase-contrast inverted microscope to examine culture flasks incubated in a 37.degree. C. (5% CO.sub.2) incubator. When the flask is placed under the phase-contrast inverted microscope, ten fields (areas on a grid inherent to the flask) are examined using the 10x objective, with the proviso that the ten fields should be non-contiguous, or significantly removed from one another, so that the ten fields are a representative sampling of the whole flask. Percentage cell occupancy for each field examined is noted, and averaging of these percentages then provides an estimate of overall percent confluency in the cell culture. When patient samples have been divided between two or among three or more flasks, an average cell count for the total patient sample should be calculated. The calculated average percent confluency should be entered into a process log to enable compilation of data--and plotting of growth curves--over time. Monolayer cultures may be photographed to document cell morphology and culture growth patterns. The applicable formula is: 1 Percent confluency = estimate of the area occupied by cells total area in an observed field

[0014] As an example, therefore, if the estimate of area occupied by the cells is 30% and the total area of the field is 100%, percent confluency is {fraction (30/100)}, or 30.

[0015] Adaptation of the above protocol for non-tumor cells is straightforward and generally constitutes an equivalent procedure.

[0016] Active agent screening using the cultured cells does not proceed in the initial incubation flask, but generally proceeds using plates such as microtiter plates. The performance of the chemosensitivity assay used for screening purposes depends on the ability to deliver a reproducible cell number to each row in a plate and/or a series of plates, as well as the ability to achieve an even distribution of cells throughout a given well. The following procedure assures that cells are reproducibly transferred from flask to microtiter plates, and cells are evenly distributed across the surface of each well.

[0017] The first step in preparing the microtiter plates is, of course, preparing and monitoring the monolayer as described above. The following protocol is exemplary and susceptible of variation as will be apparent to one skilled in the art. Cells are removed from the culture flask and a cell pellet is prepared by centrifugation. The cell pellet derived from the monolayer is then suspended in 5ml of the growth medium and mixed in a conical tube with a vortex for 6 to 10 seconds. The tube is then rocked back and forth 10 times. A 36.mu.1 droplet from the center of the conical tube is pipetted onto one well of a 96 well plate. A fresh pipette is then

used to pipette a 36 .mu.l aliquot of trypan blue solution, which is added to the same well, and the two droplets are mixed with repeated pipette aspiration. The resulting admixture is then divided between two hemocytometer chambers for examination using a standard light microscope. Cells are counted in two out of four hemocytometer quadrants, under 10x magnification. Only those cells which have not taken up the trypan blue dye are counted. This process is repeated for the second counting chamber. An average cell count per chamber is thus determined. Using means known in the art, the quadrant count values are checked, logged, multiplied by 10.sup.4to give cells/ml, and the total amount of fluid (growth medium) necessary to suspend remaining cell aliquots is calculated accordingly.

[0018] After the desired concentration of cells in medium has been determined, additional cell aliquots from the monolayer are suspended in growth medium via vortex and rocking and loaded into a Terasaki dispenser known in the art. Aliquots of the prepared cell suspension are delivered into the microtiter plates using Terasaki dispenser techniques known in the art. A plurality of plates may be prepared from a single cell suspension as needed. Plates are then wrapped in sterile wet cotton gauze and incubated in an incubator box by means known in the art.

[0019] After the microtiter plates have been prepared, exposure of the cells therein to active agent is conducted according to the following exemplary protocol. During this portion of the inventive assay, the appropriate amount of specific active agent is transferred into the microtiter plates prepared as described above. A general protocol, which may be adapted, follows. Each microtiter plate is unwrapped from its wet cotton gauze sponge and microscopically examined for cell adhesion. Control solution is dispensed into delineated rows of wells within the grid in the microtiter plate, and appropriate aliquots of active agent to be tested are added to the remaining wells in the remaining rows. Ordinarily, sequentially increasing concentrations of the active agent being tested are administered into progressively higher numbered rows in the plate. The plates are then rewrapped in their gauze and incubated in an incubator box at 37.degree. C. under 5% CO.sub.2. After a predefined exposure time, the plates are unwrapped, blotted with sterile gauze to remove the agent, washed with Hank's Balance Salt Solution, flooded with growth medium, and replaced in the incubator in an incubator box for a predefined time period, after which the plates may be fixed and stained for evaluation.

[0020] Fixing and staining may be conducted according to a number of suitable procedures; the following is representative. After removal of the plates from the incubator box, culture medium is poured off and the plates are flooded with Hank's Balance Salt Solution. After repeated flooding (with agitation each time) the plates are then flooded with reagent grade ethanol for 2-5 minutes. The ethanol is then poured off. Staining is accomplished with approximately 5 ml of Giemsa Stain per plate, although volume is not critical and flooding is the goal. Giemsa stain should be left in place 5 min..+-.30 seconds as timing influences staining intensity. The Giemsa stain is then poured off and the plates are dipped 3 times in cold tap water in a beaker. The plates are then inverted, shaken vigorously, and air dried overnight (with plate lids off) on a rack on a laboratory bench. Cells per well are then counted manually or by automated and/or computerized means, to derive data regarding chemosensitivity of cells at various concentrations of exposure. One particularly useful computer operating environment for counting cells is the commercially available OPTIMATE compiler, which is designed to permit an optical counting function well suited to computerized cell counting procedures and subsequent calculations.

[0021] The above procedures do not change appreciably when cell growth promoters are assayed rather than cell arresting agents such as chemotherapeutic agents. The present assay allows cell death or cell growth to be monitored with equal ease. In any case, optimization of use of the present system will involve the comparative testing of a variety of candidate active agents, for selection of the best candidate for patient treatment based upon the in vitro results. One particularly advantageous embodiment of the above described invention comprises a two-stage assay for cytotoxicity followed by evaluation of longer-term inhibitory effect. Chemotherapeutic agents may thus be evaluated separately for both their direct chemotherapeutic effect as well as for their longer duration efficacy.

[0022] Identification of one or more active agents or chemotherapeutic agents is peripheral to the present invention, which is intended for the efficacy screening of any or all of them as to a given patient. Literally any active agent may be screened according to the present invention; listing exemplary active agents is thus omitted

here.

[0023] The essence of the invention thus includes the important feature of the simplicity of the present system. Cohesive multicellular particulates of the patient tissue to be tested are used to form cell monolayers. Growth of those monolayers is in turn monitored for accurate prediction of correlating growth of the same cells in vivo. Finally, differing concentrations of a number of active agents may be tested for the purpose of determining not only the most appropriate agent but the most appropriate concentration of that agent for actual patient exposure (according to the calculated cell growth rates) . It is also important to note, in the context of the invention, that the present system allows in vitro tests to be conducted in suspensions of tissue culture monolayers grown in nutrient medium under fast conditions (a matter of weeks), rather than with single cell progeny produced by dilution cloning over long periods of time. In some cases, the present invention is a two stage assay for both cytotoxicity and the longer-term growth inhibitory.

[0024] It has now been determined that, despite the reliability of the above-disclosed technique to grow out only the cells of interest (malignant cells), it is additionally possible to increase the value of the associated assay with the use of staining compositions and protocols designed to characterize the malignant cells thus grown. In other words, the tissue preparation and cell culturing technique itself offers a first assurance that the cells grown out of the tumor are really the malignant tumor cells and not fibroblasts or other nonmalignant cells of no diagnostic value. As a separate confirmation, the present staining compositions and protocols offer a second, independent assurance that the cells subject to diagnostic or prognostic assay are in fact malignant cells in culture. One important characterization has to do with the nature of the malignant cells as epithelial, which is in turn an indicator of the carcinoma type of malignancy. Other characterizations of malignant cells are intended to fall within the scope of the present invention as well, although the characterization of the cells as epithelial or not is of primary importance.

[0025] The technique is practiced as follows. The same cell culturing and well distribution process is used as in the cytotoxicity assay described above, but rather than exposing the cells to chemotherapeutic or other agents, the cells are instead fixed and stained. With the stain or stain cocktail described below, the epithelial cells are identified by their intermediate filaments and/or specific membrane antigens by means of a monoclonal antibody immunoperoxidase technique. The fixative used can be any fixative which does not alter the cellular molecular markers of interest. The fixed, stained cells are then counted. If the specimen is positive for epithelial cells, the process is complete. If the specimen is negative for epithelial cells, an independent fixing and staining process is subsequently completed, with fresh cells from identical wells, using Vimentin as a stain to confirm the non-epithelial nature of the cells.

[0026] The importance of having a stain or stain cocktail (i.e., antibody cocktail), as well as an overall protocol, for identifying epithelial cells in biopsies of malignant tumors is as follows. In the basic cytotoxicity assay, the tissue culture technique is designed to grow out the cells of the tumor of origin and in fact consistently does so. Despite such reliable predictability, however, the fact that the cells of the tumor of origin did in fact grow out, and not fibroblasts or other cells, must be confirmed with independent proof before the cells can be used with complete assurance in the appropriate patient assay(s). The present technology provides a means to obtain this confirmation, which in turn furthers the interests of good laboratory and medical practice.

[0027] As a general consideration, the staining compounds or compositions of interest for use in the present technology are those which bind with cellular molecular markers unique either to epithelial or to non-epithelial cells. The invention inheres in the following two aspects: the improvement of the cytotoxicity assay by adding the epithelial staining protocol with any known epithelial stain; and the further improvement wherein specially designed stain cocktails maximize the likelihood that the presence of any known intermediate filament or specific membrane antigen, characteristic of epithelial cells, will be identified if present.

[0028] Many carcinomas are positive for any one of the intermediate filaments or specific membrane antigens characteristic of epithelial cells; virtually all if not all carcinomas are positive for one of a number of such intermediate filaments or specific membrane antigens. For example, "epithelial membrane antigen" ("EMA")

glycoproteins are known in the art and can be bound with various antiepithelial membrane antigen antibodies including monoclonal antibodies. Cytokeratin is another important epithelial cell marker and binding reagents including monoclonal antibodies are available which are specific to cytokeratin. While antisera can be raised in vivo against markers such as EMA glycoproteins and cytokeratin, as a practical matter commercially available polyclonal or monoclonal antibodies are used in the following protocols, with monoclonal antibodies being preferred.

[0029] Binding of the epithelial marker is revealed with associated staining procedures and reactions which give a visual indication that the marker binding took place. Those skilled in the art already appreciate various techniques already available--in the general field of "immunocytochemistry"--to reveal antibody-antigen reactions. One known way to accomplish this visualization when antibody binding reagents are used is with the "labeled streptavidin procedure". In this procedure, after the specimen is exposed to antibodies specific to the target antigen, a secondary "link" antibody is added. The secondary biotinylated "link" antibody consists of anti-mouse and anti-rabbit antibodies which bind universally to most primary monoclonal or polyclonal antibodies. The "link" will also connect to the tertiary reagent (peroxidase-labeled streptavidin) through chemical bonding between the biotin on the secondary reagent and the streptavidin on the streptavidin/peroxidase conjugate. Staining is completed by incubating the specimen and primary, secondary and tertiary agents in the presence of a chromagen, so that the peroxidase and the chromagen form a visible precipitate. Alternatively, a fluorescein-based detection system can be used to visualize the primary antibody, or a third alternative known in the art as the digoxigenin-conjugated detection system may be used.

[0030] Of the various epithelial markers, three have received the most widespread attention in the literature: EMA glycoproteins, cytokeratin, and carcinoembryonic antigen. In the context of this invention, the first two are the most important because literally any epithelial cell will have at least either one EMA glycoprotein on the surface thereof or a cytokeratin intermediate filament present. Therefore, the present invention resides not only in binding and staining for an epithelial marker on the surfaces of the specimen cells, but in simultaneously assaying for either or both of EMA glycoprotein(s) and cytokeratin. The cocktails of the present invention therefore contain binding reagents for both EMA glycoproteins and cytokeratin and, importantly, are selected to include the most generally applicable binding reagents in combination so that the cocktail has the broadest binding scope possible. The cocktails identified in Examples 1 and 2, for example, represent a combination of two general binding reagents (containing a total of three monoclonal antibodies) for cytokeratin, admixed with a general binding reagent for EMA glycoprotein. The dual benefit of this admixture of general binding agents is that the incidence of false negatives for epithelial cells is minimized, and the visible staining reactions are generally stronger when the combined binding reagents are used in lieu of a single binding reagent.

[0031] Although the binding reagents and other reagents identified in the Examples are the preferred reagents for use in the context of the invention, the invention is intended to encompass epithelial-specific binding and staining reagents generally. These include, without limitation: Boehringer-Mannheim AE1 anti-cytokeratin antibody; Boehringer-Mannheim AE3 anti-cytokeratin antibody; Boehringer-Mannheim AE1/AE3 anti-cytokeratin antibody (AE1 and AE3 in admixture); Becton-Dickinson CAM 5.2 antibody, DAKO EMA antibody, Biomeda's Anti-Cytokeratin Cocktail CK22, Biomeda's Anti-Cytokeratin Cocktail CK23, Biomeda's Anti-Pan-Cytokeratin CK56, Biomeda's polyclonal goat or rabbit anti-cytokeratin antisera, ScyTek Laboratories' anti-EMA antigen antibody clone E29, and many others. Those skilled in the art and in possession of the guidance provided herein can readily determine alternative, equivalent binding and staining reagents and cocktails, to accomplish the disclosed result. These binding agents and cocktails may be used in combination with any known visualization system, such as the streptavidin, fluorescein- and digoxigenin-conjugated systems identified above.

[0032] As a control, Vimentin antibody is used as a binding alternative either in conjunction with binding and staining of the test cells, or subsequently thereto. In the context of this invention, Vimentin can be considered a binding reagent which is specific to non-epithelial cells.

[0033] The following examples are illustrative.

Example 1

[0034] A tumor biopsy of approximately 100 mg of non-necrotic, non-contaminated tissue was harvested from the patient by surgical biopsy and transferred to the laboratory in a standard shipping container. Biopsy sample preparation proceeded as follows. Reagent grade ethanol was used to wipe down the surface of a Laminar flow hood. The tumor was then removed, under sterile conditions, from its shipping container, and cut into quarters with a sterile scalpel. Using sterile forceps, each undivided tissue quarter was then placed in 3 ml sterile growth medium (Standard F-10 medium containing 17% calf serum and a standard amount of Penicillin and Streptomycin) and was systematically minced by using two sterile scalpels in a scissor-like motion. The tumor particulates each measured about 1 mm.sup.3. After each tumor quarter was minced, the particles were plated in culture flasks using sterile pasteur pipettes (9 explants per T-25 or 20 particulates per T-75 flask). Each flask was then labeled with the patient's code, the date of explanation and any other distinguishing data. The explants were evenly distributed across the bottom surface of the flask, with initial inverted incubation in a 37.degree. C. incubator for 5-10 minutes, followed by addition of about 5-10ml sterile growth medium and further incubation in the normal, non-inverted position. Flasks were placed in a 35.degree. C., non-CO.sub.2 incubator. Flasks were checked daily for growth and contamination. Over a period of a few weeks, with weekly removal and replacement of 5ml of growth medium, the explants grew out into a monolayer.

[0035] The cells were subsequently removed from the culture flask and a cell pellet was prepared by centrifugation. The cell pellet derived from the monolayer was then suspended in 5ml of the growth medium and mixed in a conical tube with a vortex for 6 to 10 seconds. The tube was then rocked back and forth 10 times. A 36 .mu.l droplet from the center of the conical tube was pipetted onto one well of a 96 well plate. A fresh pipette was then used to pipette a 36 .mu.l aliquot of trypan blue solution, which was added to the same well, and the two droplets were mixed with repeated pipette aspiration. The resulting admixture was then divided between two hemocytometer chambers for examination using a standard light microscope. Cells were counted in two out of four hemocytometer quadrants, under 10x magnification. Only those cells which had not taken up the trypan blue dye were counted. This process was repeated for the second counting chamber. An average cell count per chamber was thus determined. Using means known in the art, the quadrant count values were checked, logged, multiplied by 10.sup.4 to give cells/ml, and the total amount of fluid (growth medium) necessary to suspend remaining cell aliquots was calculated accordingly.

[0036] After the desired concentration of cells in medium was determined, additional cell aliquots from the monolayer were suspended in growth medium via vortex and rocking, and were loaded into a Terasaki dispenser known in the art. Aliquots of the prepared cell suspension were delivered into the microtiter plates using Terasaki dispenser techniques known in the art. A plurality of plates were prepared from a single cell suspension. Plates were then wrapped in sterile wet cotton gauze and incubated in an incubator box overnight, after which the microtiter wells were examined to assure that the cells had settled onto and adhered to each well base.

[0037] One microtiter plate was selected and segregated from the others intended for cytotoxicity assay, and the cells were examined as follows. Each well was overlaid with an aliquot of 95% ethanol for five minutes, 70% ethanol for five minutes, and 30% hydrogen peroxide/70% methanol for thirty minutes. Each well was then rinsed in Tris-saline several times.

[0038] Saving the first and last rows in the multi-well plate as controls, the remaining wells were each inoculated with approximately 8 microliters of an antibody cocktail including 80 microliters CAM 5.2 antibody (Becton-Dickinson), 10 microliters of a 1:30 dilution of AE1/AE3 (Boehringer-Mannheim) and 10 microliters of a 1:10 dilution of EMA antibody (DAKO). The wells were incubated at room temperature for one hour and were then rinsed in several changes of Tris-saline. Using an LSAB (streptavidin) kit, about 8 microliters of the secondary reagent (in the DAKO LSAB2 kit, the secondary reagent is yellow) was added to each well, followed by a one hour room-temperature incubation and several rinses in Tris-saline. The tertiary reagent (pink) was then inoculated in approximately 8 microliter amounts to each well, followed by a one hour room-temperature incubation followed by rinsing. An overlayer of Tris-saline was retained in each well while fresh filtered diaminobenzidine tetrahydrochloride (DAB)/H2O2 solution was prepared. The Tris-saline was discarded from each well and replaced with DAB solution. After a

five minute room-temperature incubation, the cells were rinsed in distilled water, stained in hematoxylin for between 30 seconds and 1 minute, rinsed in warm running tap water for 2-5 minutes and rinsed in distilled water.

[0039] In the rows saved for use as a control, the above protocol was repeated except that the antibody cocktail was replaced with 8 microliters 1:200 dilution Vimentin antibody, with every other step being the same.

[0040] For the test wells, a strong brown stain upon visual inspection confirmed the identity of the cells as epithelial cells. Consistent with this observation, all the wells in the control rows were negative.

Example 2

[0041] Example 1 was repeated except that in the test wells an alternate cocktail of binding reagents was used containing 60 microliters CAM 5.2, 20 microliters of a 1:30 dilution of AE1/AE3, and 20 microliters of a 1:10 dilution of EMA antibody. The test wells all stained positive (brown) for epithelial cells; the control wells were negative for non-epithelial cells.

[0042] Although the present invention has been described with respect to specific materials and methods above, the invention is only to be considered limited insofar as is set forth in the accompanying claims. For example, although solid tumors have been discussed above, any malignant cells including but not limited to blood, lymph and other cells may be subjected to the present protocols.

CLAIMS:

We claim:

1. A method for assessing sensitivity of patient cells comprising the steps of: a) obtaining a malignant tissue specimen; b) separating said specimen into multicellular particulates; c) growing a tissue culture monolayer from said cohesive multicellular particulates; d) inoculating cells from said monolayer into a plurality of segregated sites; e) binding and staining some of said plurality of sites with a staining protocol including at least a staining protocol including one binding agent specific for epithelial cells; f) treating the remainder of said plurality of sites with at least one agent; g) examining said plurality of sites; and h) assessing sensitivity of the cells in said remainder of said plurality of sites.

2. The method according to claim 1 wherein said plurality of segregated sites comprise a plate containing a plurality of wells therein.

3. The method according to claim 2 wherein said binding agent specific for epithelial cells includes at least one of anti-cytokeratin antibody and anti-epithelial-membrane-antigen antibody.

4. The method according to claim 3 wherein said binding agent specific for epithelial cells includes both anti-cytokeratin antibody and anti-epithelial-membrane-antigen antibody and further wherein the binding resulting from the addition of the binding agent is made visible via a chemical reaction with a streptavidin/peroxidase conjugate and a chromagen.

5. An antibody cocktail containing at least two monoclonal antibodies specific to cytokeratin and at least one monoclonal antibody specific to epithelial membrane antigen.

6. An antibody cocktail containing 80 microliters CAM 5.2 antibody (Becton-Dickinson), 10 microliters of a 1:30 dilution of AE1/AE3 (Boehringer-Mannheim) and 10 microliters of a 1:10 dilution of EMA antibody (DAKO).

7. An antibody cocktail containing 60 microliters CAM 5.2 antibody (Becton-Dickinson), 20 microliters of a 1:30 dilution of AE1/AE3 (Boehringer-Mannheim), and 20 microliters of a 1:10 dilution of EMA antibody (DAKO).

8. A method of confirming the epithelial character of malignant cells grown in culture, for use in a subsequent sensitivity assay, comprising binding a sample of said malignant cells with at least one binding agent specific for epithelial cell

markers, conducting a chemical reaction with said binding reagent to render visible any resultant binding and to confirm the epithelial character of the cells, and subjecting fresh samples of the same malignant cells to sensitivity assays in vitro.

9. A method of confirming the epithelial character of malignant cells grown in culture, for use in a subsequent sensitivity assay, comprising binding a sample of said malignant cells with at least one binding agent further comprising an antibody cocktail containing at least two anti-cytokeratin monoclonal antibodies and at least one anti-epithelial membrane antigen monoclonal antibody, conducting a chemical reaction with said binding reagent to render visible any resultant binding, and subjecting fresh samples of the same malignant cells to sensitivity assays in vitro.

10. A method of confirming the epithelial character of malignant cells grown in culture, for use in a subsequent sensitivity assay, comprising binding a sample of said malignant cells with at least one binding agent further comprising an antibody cocktail containing 80 microliters CAM 5.2 antibody (Becton-Dickinson), 10 microliters of a 1:30 dilution of AE1/AE3 (Boehringer-Mannheim) and 10 microliters of a 1:10 dilution of EMA antibody (DAKO), conducting a chemical reaction with said binding reagent to render visible any resultant binding, and subjecting fresh samples of the same malignant cells to sensitivity assays in vitro.

11. A method of confirming the epithelial character of malignant cells grown in culture, for use in a subsequent sensitivity assay, comprising binding a sample of said malignant cells with at least one binding agent further comprising an antibody cocktail containing 60 microliters CAM 5.2 antibody (Becton-Dickinson), 20 microliters of a 1:30 dilution of AE1/AE3 (Boehringer-Mannheim), and 20 microliters of a 1:10 dilution of EMA antibody (DAKO), conducting a chemical reaction with said binding reagent to render visible any resultant binding, and subjecting fresh samples of the same malignant cells to sensitivity assays in vitro.

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ABSTRACT:

An improved system for screening a multiple of candidate therapeutic or chemotherapeutic agents for efficacy as to a specific patient, in which a tissue sample from the patient is harvested, cultured and separately exposed to a plurality of treatments and/or therapeutic agents for the purpose of objectively identifying the best treatment or agent for the particular patient. Specific method innovations such as tissue sample preparation techniques render this method practically as well as theoretically useful. One particularly important tissue sample preparation technique is the initial preparation of cohesive multicellular particulates of the tissue sample, rather than enzymatically dissociated cell suspensions or preparations, for initial tissue culture monolayer preparation. With respect to the culturing of malignant cells, for example, it is believed (without any intention of being bound by the theory) that by maintaining the malignant cells within a multicellular particulate of the originating tissue, growth of the malignant cells themselves is facilitated versus the overgrowth of fibroblasts or other cells which tends to occur when suspended tumor cells are grown in culture. Practical monolayers of cells may thus be formed to enable meaningful screening of a plurality of treatments and/or agents. Growth of cells is monitored to ascertain the time to initiate the assay and to determine the growth rate of the cultured cells; sequence and timing of drug addition is also monitored and optimized. By subjecting uniform samples of cells to a wide variety of active agents (and concentrations thereof), the most promising agent and concentration for treatment of a particular patient can be determined. For assays concerning cancer treatment, a two-stage evaluation is contemplated in which both acute cytotoxic and longer term inhibitory effect of a

given anti-cancer agent are investigated.

FIELD OF THE INVENTION

[0001] The invention relates to screening and testing of active agents, including chemotherapeutic agents, to predict potential efficacy in individual patients in whom treatment with such agents is indicated.

INTRODUCTION

[0002] All active agents including chemotherapeutic active agents are subjected to rigorous testing as to efficacy and safety prior to approval for medical use in the United States. Methods of assessing efficacy have included elaborate investigations of large populations in double blind studies as to a given treatment method and/or active agent, with concommitant statistical interpretation of the resulting data, but these conclusions are inevitably generalized as to patient populations taken as a whole. In many pharmaceutical disciplines and particularly in the area of chemotherapy, however, the results of individual patient therapy may not comport with generalized data--to the detriment of the individual patient. The need has been long recognized for a method of assessing the therapeutic potential of active agents, including but not limited to chemotherapeutic agents, for their efficacy as to a given individual patient, prior to the treatment of that patient.

[0003] Prior art assays already exist which expose malignant tissue of various types to a plurality of active agents, for the purpose of assessing the best choice for therapeutic administration. For example, in Kruczynski, A., et al., "Evidence of a direct relationship between the increase in the in vitro passage number of human non-small-cell-lung cancer primocultures and their chemosensitivity," Anticancer Research, vol. 13, no. 2, pp. 507-513 (1993), chemosensitivity of non-small-cell-lung cancers was investigated in in vivo grafts, in in vitro primocultures and in commercially available long-term cancer cell lines. The increase in chemosensitivity was documented and correlated with morphological changes in the cells in question. Sometimes animal model malignant cells and/or established cell cultures are tested with prospective therapy agents, see for example Arnold, J. T., "Evaluation of chemopreventive agents in different mechanistic classes using a rat tracheal epithelial cell culture transformation assay," Cancer Res., vol. 55, no. 3, pp. 537-543 (1995).

[0004] When actual patient cells are used to form in vitro assays focussed on individual patients, in typical prior art processes the cells are harvested (biopsied) and trypsinized (connective tissue digested with the enzyme trypsin) to yield a cell suspension suitable for conversion to the desired tissue culture form. The in vitro tissue culture cell collections which result from these techniques are generally plagued by their inability accurately to imitate the chemosensitivity of the original tumor or other cell biopsy. Standard cloning and tissue culture techniques are moreover excessively complicated and expensive for use in a patient-by-patient assay setting. A need thus remains for a technique of tissue culture preparation which provides cell cultures, for drug screening purposes, in which after simple preparation the cell cultures react in a manner equivalent to their in vivo reactivity, to enable drug or chemotherapeutic agent screening as to a particular patient for whom such screening is indicated.

SUMMARY OF THE INVENTION

[0005] In order to meet this need, the present invention is an improved system for screening a multiple of candidate therapeutic or chemotherapeutic agents for efficacy as to a specific patient, in which a tissue sample from the patient is harvested, cultured and separately exposed to a plurality of treatments and/or therapeutic agents for the purpose of objectively identifying the best treatment for the cultured cells obtained from the patient. Specific method innovations such as tissue sample preparation techniques render this method practically as well as theoretically useful. One particularly important tissue sample preparation technique is the initial preparation of cohesive multicellular particulates of the tissue sample, rather than enzymatically dissociated cell suspensions or preparations, for initial tissue culture monolayer preparation. With respect to the culturing of malignant cells, for example, it is believed (without any intention of being bound by the theory) that by maintaining the malignant cells within a multicellular particulate of the originating tissue, growth of the malignant cells themselves is facilitated versus the overgrowth of fibroblasts or other cells which tends to occur

when suspended tumor cells are grown in culture. Practical monolayers of cells may thus be formed to enable meaningful screening of a plurality of treatments and/or agents. Growth of cells is monitored to ascertain the time to initiate the assay and to determine the growth rate of the cultured cells; sequence and timing of drug addition is also monitored and optimized. By subjecting uniform samples of cells to a wide variety of active agents (and concentrations thereof), the most efficacious agent can be determined. For assays concerning cancer treatment, a two-stage evaluation is contemplated in which both acute cytotoxic and longer term inhibitory effect of a given anti-cancer agent are investigated.

DETAILED DESCRIPTION OF THE INVENTION

[0006] The present invention is a system for screening a multiple of candidate therapeutic or chemotherapeutic agents for efficacy as to a specific patient, in which a tissue sample from the patient is harvested and separately exposed to a plurality of treatments and/or therapeutic agents for the purpose of objectively identifying the best treatment or agent. Specific method innovations such as tissue sample preparation techniques render this method practically as well as theoretically useful. One particularly important tissue sample preparation technique is the initial preparation of cohesive multicellular particulates (explants) of the tissue sample, rather than enzymatically dissociated cell suspensions or preparations, for initial tissue culture monolayer preparation. Cell growth, and sequence and timing of drug addition, are monitored and optimized.

[0007] An important application of the present invention is the screening of chemotherapeutic agents and other antineoplastic therapies against tissue culture preparations of malignant cells from the patients from whom malignant samples are biopsied. Related anti-cancer therapies which can be screened using the inventive system are both radiation therapy and agents which enhance the cytotoxicity of radiation, as well as immunotherapeutic anti-cancer agents. Screening processes for treatments or therapeutic agents for nonmalignant syndromes are also embraced within this invention, however, and include without limitation agents which combat hyperproliferative syndromes, such as psoriasis, or wound healing agents. Nor is the present efficacy assay limited only to the screening of active agents which speed up (healing) or slow down (anti-cancer, anti-hyperproliferative) cell growth because agents intended to enhance or to subdue intracellular biochemical functions may be tested in the present tissue culture system also. For example, the formation or blocking of enzymes, neurotransmitters and other biochemicals may be screened with the present assay methods prior to treatment of the patient.

[0008] When the patient is to be treated for the presence of tumor, in the preferred embodiment of the present invention a tumor biopsy of>100 mg of non-necrotic, non-contaminated tissue is harvested from the patient by any suitable biopsy or surgical procedure known in the art. Biopsy sample preparation generally proceeds as follows under a Laminar Flow Hood which should be turned on at least 20 minutes before use. Reagent grade ethanol is used to wipe down the surface of the hood prior to beginning the sample preparation. The tumor is then removed, under sterile conditions, from the shipping container and is minced with sterile scissors. If the specimen arrives already minced, the individual tumor pieces should be divided into four groups. Using sterile forceps, each undivided tissue quarter is then placed in 3 ml sterile growth medium (Standard F-10 medium containing 17% calf serum and a standard amount of Penicillin and Streptomycin) and systematically minced by using two sterile scalpels in a scissor-like motion, or mechanically equivalent manual or automated opposing incisor blades. This cross-cutting motion is important because the technique creates smooth cut edges on the resulting tumor multicellular particulates. Preferably but not necessarily, the tumor particulates each measure 1 mm.sup.3. After each tumor quarter has been minced, the particles are plated in culture flasks using sterile pasteur pipettes (9 explants per T-25 or 20 particulates per T-75 flask). Each flask is then labelled with the patient's code, the date of explantation and any other distinguishing data. The explants should be evenly distributed across the bottom surface of the flask, with initial inverted incubation in a 37.degree. C. incubator for 5-10 minutes, followed by addition of about 5-10 ml sterile growth medium and further incubation in the normal, non-inverted position. Flasks are placed in a 35.degree. C., non-CO.sub.2 incubator. Flasks should be checked daily for growth and contamination. Over a period of a few weeks, with weekly removal and replacement of 5 ml of growth medium, the explants will foster growth of cells into a monolayer. With respect to the culturing of malignant cells, it is believed (without any intention of being bound by the theory) that by maintaining the malignant cells within a multicellular particulate of the

originating tissue, growth of the malignant cells themselves is facilitated versus the overgrowth of fibroblasts (or other unwanted cells) which tends to occur when suspended tumor cells are grown in culture.

[0009] The use of the above procedure to form a cell monolayer culture maximizes the growth of malignant cells from the tissue sample, and thus optimizes ensuing tissue culture assay of chemotherapeutic action of various agents to be tested. Enhanced growth of actual malignant cells is only one aspect of the present invention, however; another important feature is the growth rate monitoring system used to oversee growth of the monolayer once formed. Once a primary culture and its derived secondary monolayer tissue culture has been initiated, the growth of the cells is monitored to ascertain the time to initiate the chemotherapy assay and to determine the growth rate of the cultured cells.

[0010] Monitoring of the growth of cells is conducted by counting the cells in the monolayer on a periodic basis, without killing or staining the cells and without removing any cells from the culture flask. The counting may be done visually or by automated methods, either with or without the use of estimating techniques known in the art (counting in a representative area of a grid multiplied by number of grid areas, for example). Data from periodic counting is then used to determine growth rates which may or may not be considered parallel to growth rates of the same cells in vivo in the patient. If growth rate cycles can be documented, for example, then dosing of certain active agents can be customized for the patient. The same growth rate can be used to evaluate radiation treatment periodicity, as well. It should be noted that with the growth rate determinations conducted while the monolayers grow in their flasks, the present method requires no hemocytometry, flow cytometry or use of microscope slides and staining, with all their concomitant labor and cost.

[0011] Protocols for monolayer growth rate generally use a phase-contrast inverted microscope to examine culture flasks incubated in a 37.degree. C. (5% CO.sub.2) incubator. When the flask is placed under the phase-contrast inverted microscope, ten fields (areas on a grid inherent to the flask) are examined using the 10.times. objective, with the proviso that the ten fields should be non-contiguous, or significantly removed from one another, so that the ten fields are a representative sampling of the whole flask. Percentage cell occupancy for each field examined is noted, and averaging of these percentages then provides an estimate of overall percent confluency in the cell culture. When patient samples have been divided between two or among three or more flasks, an average cell count for the total patient sample should be calculated. The calculated average percent confluency should be entered into a process log to enable compilation of data--and plotting of growth curves--over time. Monolayer cultures may be photographed to document cell morphology and culture growth patterns. The applicable formula is: 1 Percent confluency = estimate of the area occupied by cells total area in an observed field

[0012] As an example, therefore, if the estimate of area occupied by the cells is 30% and the total area of the field is 100%, percent confluency is 30/100, or 30.

[0013] Adaptation of the above protocol for non-tumor cells is straightforward and generally constitutes an equivalent procedure.

[0014] Active agent screening using the cultured cells does not proceed in the initial incubation flask, but generally proceeds using plates such as microtiter plates. The performance of the chemosensitivity assay used for screening purposes depends on the ability to deliver a reproducible cell number to each row in a plate and/or a series of plates, as well as the ability to achieve an even distribution of cells throughout a given well. The following procedure assures that cells are reproducibly transferred from flask to microtiter plates, and cells are evenly distributed across the surface of each well.

[0015] The first step in preparing the microtiter plates is, of course, preparing and monitoring the monolayer as described above. The following protocol is exemplary and susceptible of variation as will be apparent to one skilled in the art. Cells are removed from the culture flask and a cell pellet is prepared by centrifugation. The cell pellet derived from the monolayer is then suspended in 5 ml of the growth medium and mixed in a conical tube with a vortex for 6 to 10 seconds. The tube is then rocked back and forth 10 times. A 36 .mu.l droplet from the center of the conical tube is pipetted onto one well of a 96 well plate. A fresh pipette is then used to pipette a 36 .mu.l aliquot of trypan blue solution, which is added to the same well, and the two droplets are mixed with repeated pipette aspiration. The

resulting admixture is then divided between two hemocytometer chambers for examination using a standard light microscope. Cells are counted in two out of four hemocytometer quadrants, under 10.times. magnification. Only those cells which have not taken up the trypan blue dye are counted. This process is repeated for the second counting chamber. An average cell count per chamber is thus determined. Using means known in the art, the quadrant count values are checked, logged, multiplied by 10.sup.4 to give cells/ml, and the total amount of fluid (growth medium) necessary to suspend remaining cell aliquots is calculated accordingly.

[0016] After the desired concentration of cells in medium has been determined, additional cell aliquots from the monolayer are suspended in growth medium via vortex and rocking and loaded into a Terasaki dispenser known in the art. Aliquots of the prepared cell suspension are delivered into the microtiter plates using Terasaki dispenser techniques known in the art. A plurality of plates may be prepared from a single cell suspension as needed. Plates are then wrapped in sterile wet cotton gauze and incubated in an incubator box by means known in the art.

[0017] After the microtiter plates have been prepared, exposure of the cells therein to active agent is conducted, according to the following exemplary protocol. During this portion of the inventive assay, the appropriate amount of specific active agent is tranferred into the microtiter plates prepared as described above. A general protocol, which may be adapted, follows. Each microtiter plate is unwrapped from its wet cotton gauze sponge and microscopically examined for cell adhesion. Control solution is dispensed into delineated rows of wells within the grid in the microtiter plate, and appropriate aliquots of active agent to be tested are added to the remaining wells in the remaining rows. Ordinarily, sequentially increasing concentrations of the active agent being tested are administered into progressively higher numbered rows in the plate. The plates are then rewrapped in their gauze and incubated in an incubator box at 37.degree. C. under 5% CO.sub.2. After a predefined exposure time, the plates are unwrapped, blotted with sterile gauze to remove the agent, washed with Hank's Balance Salt Solution, flooded with growth medium, and replaced in the incubator in an incubator box for a predefined time period, after which the plates may be fixed and stained for evaluation.

[0018] Fixing and staining may be conducted according to a number of suitable procedures; the following is representative. After removal of the plates from the incubator box, culture medium is poured off and the plates are flooded with Hank's Balance Salt Solution. After repeated flooding (with agitation each time) the plates are then flooded with reagent grade ethanol for 2-5 minutes. The ethanol is then poured off. Staining is accomplished with approximately 5 ml of Giemsa Stain per plate, although volume is not critical and flooding is the goal. Giemsa stain should be left in place 5 min..+-.30 seconds as timing influences staining intensity. The Giemsa stain is then poured off and the plates are dipped 3 times cold tap water in a beaker. The plates are then inverted, shaken vigorously, and air dried overnight (with plate lids off) on a rack on a laboratory bench. Cells per well are then counted manually or by automated and/or computerized means, to derive data regarding chemosensitivity of cells at various concentrations of exposure. One particularly useful computer operating environment for counting cells is the commercially available OPTIMATE compiler, which is designed to permit an optical counting function well suited to computerized cell counting procedures and subsequent calculations.

[0019] The above procedures do not change appreciably when cell growth promoters are assayed rather than cell arresting agents such as chemotherapeutic agents. The present assay allows cell death or cell growth to be monitored with equal ease. In any case, optimization of use of the present system will involve the comparative testing of a variety of candidate active agents, for selection of the best candidate for patient treatment based upon the in vitro results. One particularly advantageous embodiment of the above described invention comprises a two-stage assay for cytotoxicity followed by evaluation of longer-term inhibitory effect. Chemotherapeutic agents may thus be evaluated separately for both their direct chemotherapeutic effect as well as for their longer duration efficacy.

[0020] Identification of one or more active agents or chemotherapeutic agents is peripheral to the present invention, which is intended for the efficacy screening of any or all of them as to a given patient. Literally any active agent may be screened according to the present invention; listing exemplary active agents is thus omitted here.

[0021] The essence of the invention thus includes the important feature of the simplicity of the present system--cohesive multicellular particulates of the patient tissue to be tested are used to form cell monolayers; growth of those monolayers is monitored for accurate prediction of correlating growth of the same cells in vivo; and differing concentrations of a number of active agents may be tested for the purpose of determining not only the most appropriate agent but the most appropriate concentration of that agent for actual patient exposure (according to the calculated cell growth rates). It is also important to note, in the context of the invention, that the present system allows in vitro tests to be conducted in suspensions of tissue culture monolayers grown nutrient medium under fast conditions (a matter of weeks), rather than with single cell progeny produced by dilution cloning over long periods of time. In some cases, the present invention is a two stage assay for both cytotoxicity and the longer-term growth inhibitory.

[0022] Although the present invention has been described with respect to specific materials and methods above, the invention is only to be considered limited insofar as is set forth in the accompanying claims.

CLAIMS:

I claim:

1. A method for assessing chemosensitivity of patient cells comprising the steps of: a) harvesting a specimen of a patient's tissue, cells ascites, or effusion fluid; b) separating said specimen into multicellular particulates; c) growing a tissue culture monolayer from said cohesive multicellular particulates; d) inoculating cells from said monolayer into a plurality of segregated sites; and e) treating said plurality of sites with at least one active agent, followed by assessment of chemosensitivity of the cells in said site to at least one active agent.

2. The method according to claim 1 wherein step a) further comprises the step of a) preparing a specimen which was harvested from a sample of patient tumor tissue;.

3. The method according to claim 1 wherein said plurality of segregated sites further comprises a plate containing a plurality of wells therein.

4. The method according to claim 1 wherein step e) further comprises the step of: e) treating said plurality of sites with a plurality of active agents at varied concentrations, followed by assessment of optimal chemosensitivity with respect to a single active agent at a single concentration.

5. The method according to claim 1 wherein step e) further comprises the step of: e) treating said plurality of sites with a plurality of active agents over a length of time adequate to permit assessment of both initial cytotoxic effect and longer-term inhibitory effect of at least one of said plurality of active agents.

6. The method according to claim 1 wherein the chemosensitivity assayed according to step e) is anti-cancer sensitivity.

7. The method according to claim 1 wherein step d) is accomplished using a Terasaki dispenser.

8. The method according to claim 1 wherein the cells in step d) are prepared in suspension prior to inoculation into a plurality of wells in a culture plate.

9. The method according to claim 1, wherein said active agent is a chemotherapeutic agent.

10. The method according to claim 1, wherein said active agent is a wound healing agent.

11. The method according to claim 1, wherein said active agent is a radiation therapy and/or a radiation therapy sensitizing or ameliorating agent.

12. The method according to claim 1, where said active agent is an immunotherapeutic agent.